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BEHAVIOUR OF EXPERIMENTAL PULMONARY TUBERCULOUS CAVITIES FOLLOWING INTRAVENOUS ADMINISTRATION OF FAT EMULSION

by

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I INTRODUCTION

It has been almost definitely established by many investigators, such as Dettweiler, Weigert, Schröder, Kumagai, Omori, Haramura, Iwazuru, etc. that "fat nutrition" is somewhat effective against tuberculosis. It has been made clear by the results of the histopathologic or biochemical investigations of Rosenthal, Hagemeister, Herxheimer, Joest, Caldwell, Wells, etc. that the caseous debris of tuberculous tissue is rich in fatty substances.

If it is true that "fat nutrition" is effective against tuberculosis what is the mechanism of action? Why are fatty substances concentrated in the central part of small tuberculous lesions, and in the borders of the larger lesions. Furthermore, why are they arranged in layers like concentric circles in the caseous zone?

The answers to the above questions are still obscure. The reasons that these have not been investigated much, despite of the close connection between fatty substances and tuberculosis, may be as follows:

First, the study of the fatty substances themselves has lagged behind that of protein and carbohydrate. Second, induction of experimental tuberculosis, particulary cavity formation in animals which then survive for a long period has been very difficult by the methods used in the past.

Recently a fat emulsion capable of being safely injected intravenously has been made by Hikasa in our laboratory. The emulsion itself acts upon the living body

as a strongly stimulating agent, as well as providing one new means to be added to the methods already in use of investigating fat metabolism.

Moreover, OGAWA & OKAMOTO have succeeded in producing experimental tuberculous cavities mainly in the lungs of dogs by their pertracheobronchial infection method under fluoroscopy. By modifying this method a little, the writer has almost always produced tuberculous cavities in the lungs of rabbits, which have subsequently survived for a fairly long period.

The writer has tried directly or indirectly to answer the question, how has fat emulsion, used for daily intravenous injection, acted on pulmonary tuberculosis of rabbits, particularly on cavities? The histochemical findings of the cavity wall, in addition to observations upon the change of body weight, the chest roentgenologic examinations, etc., have been especially studied.

II MATERIAL AND METHOD

1) FAT EMULSION AND THE METHOD OF INJECTION: Male rabbits weighing about 1.5 kg were injected intravenously with 1.5 cc of 15% sesame oil emulsion daily at a fixed time, to which was added 3.75 mg of l-methionine and a quantity of riboflavine equivalent to 0.75 mg of vitamin B_2 phosphate per kg of body weight.

2) FINELY DIVIDED CARBON PARTICLES AND THE METHOD OF INJECTION: Even if the infused fat particles reach the sites of tuberculous lesions, it is hard to distinguish by Sudan III stain the difference between the particles and the fatty substances originally included in the lesions.

MENKIN and other investigators indicated that foreign particles introduced into blood vessels could be accumulated in inflammatory foci or could escape and penetrate into tissue from the diseased vessels. Although particles of fat and carbon are of different character, they are similar in being foreign bodies. Therefore, the writer considered that if they were injected intravenously at the same time, the carbon which is easily recognized histologically might represent indirectly the appearance of the fat as it penetrates into a lesion or is distributed in the cavity wall. A 5% solution of finely divided carbon particles (KOKABOKU, KOBAIEN) was filtered twice, and the filtrate was sterilized and used for intravenous injection. Following the studies of TERADA et al., the diameter of the carbon particle ranged from 120$\mu\mu$ to 220$\mu\mu$.

In comparison with the findings by YASUHIRA and YAMAMOTO, a few of the rabbits injected with fat emulsion for a long period were sacrificed 45 minutes after the last injection, which was done with 15 cc of the above carbon solution per kg of body weight.

Control rabbits were treated intravenously with the carbon solution alone, which had been diluted with enough sterile distilled water to equal the volume of fat emulsion, calculated from their body weight, received by the experimental rabbits.

3) SUSPENSION OF TUBERCLE BACILLI: Because of the characteristics

of this experiment, oily contrast drugs for fluoroscopy could not be used. So, a larger number of tubercle bacilli had to be suspended in some non-oily contrast material of as high viscosity and as little volume as possible.

After considerable investigation, barium phosphate was chosen, and amounts suitable for roentgenologic visualization were added to the optimal amount of APATHY'S gummy syrup used for enclosing histologic specimens, and the two were mixecd thoroughly. Finally, the viscosity of the mixture was regulated to bring it close to that of an oily X-ray contrast drug (MOLJODOL).

Next, a calculated amount (dry weight) of the surface growth of virulent H37 Rv strain of M. tuberculosis var. hominis on SAUTON'S culture medium was added to a fixed quantity of this X-ray contrast mixture and mixed thoroughly with it. In oorder to determine the suitable quantity of tubercle bacilli to inject into rabbits for cavity formation, 0.5～20.0 mg of H37 Rv strain of human type tubercle bacilli was mixed with 0.25 cc of the X-ray contrast mixture. 10～18 mg of the strain was found to be successful in producing cavities.

4) BRONCHIAL CATHETER FOR INFUSING BACILLI: For the purpose of simplifying this experiment during which much fluoroscopy is done in the dark room, and of decreasing infection and contamination by tubercle bacilli, a special bronchial catheter, shown in Fig. 1, was constructed by a modification of the method of OGAWA and his co-worker. It consists of the following three main parts.

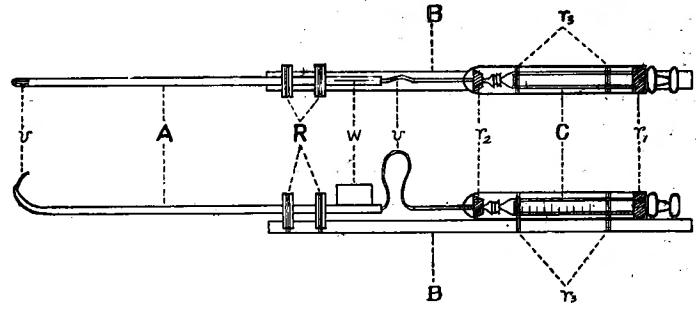


Fig. 1. Diagram of bronchial catheter for infusing tubercle bacilli. Dorsal view (upper) and side view (lower).

The first (A) is a metal catheter, measuring 2.5 mm on the outer, 2.0 mm on the inner diameter, and 27 cm in length, having a small ellipsoid aperture (2mm × 2mm × 4mm) at its curved tip, the long axis of which is parallel to that of the catheter. At the other end there is a direction-index-wing (w) placed on the same side as the aperture. A vinyl tube with an outside diameter of 1.5 mm, and about 35 cm in length, is inserted in the catheter. The catheter penetrates 2 rubber stoppers (R) which are fastened by wire to part (B).

The second part (B) is a wooden splint, 5 mm in height, 7 mm in width and 24 mm in length.

The third part (C) is an injection-syringe, placed inside a hard glass test-tube. It is fixed to the tube through a large-holed rubber stopper (r_1). The vinyl tube (v) is passed through a close-fitting opening at the bottom of the glass tube, and joined to an injection needle. A second rubber stopper (r_2) around the needle keeps the syringe steady. (C) is fixed to (B) with two rubber bands (r_3) in any

position such that (C), as a whole, may be slid smoothly back and forth along the long axis of (B).

Since (v) is too soft to be introduced into a bronchiole, it is repeatedly boiled and cooled alternately until it is stiff enough to maintain almost a straight line when one end is held. When the end of (v) is hardened it is bent like a fish-hook by hand, and can easily be introduced into an upper lobe.

After sterilization of the entire instrument by boiling, the injector, removed with (r_1) from the glass tube, is filled with a certain quantity of the suspension of tubercle bacilli mentioned above. The injector is joined to the injection-needle again and the bacillary suspension is first poured as far as the end of (v). After the whole (C) is returned to its former position, the end of (v) is kept within (A).

5) EXPERIMENTAL GROUPS AND OBSERVATION METHODS: The rabbits were divided into four groups (Table I; K(F), K(O), G(F), G(O).). Each of these was divided again into two subgroups according to postoperative observation of 6 months or 8 months, and was marked as follows; the former……$K(F)_6$, $K(O)_6$, $G(F)_6$, $G(O)_6$; the latter ……$K(F)_8$, $K(O)_8$, $G(F)_3$, $G(O)_8$: all these marks will be used below in this report.

Obsevations were carried out on them, and the groups were compared mainly as to histochemical changes of the cavity wall or surrounding area, and also as to general conditions including changes of body weight, periodical chest roentgenogram, gross specimens and roentgenogram of removed lungs. Additional observations were also made to determine whether this fat emulsion, given over a prolonged period, would produce any side actions.

6) METHOD OF OPERATION FOR PERFORMING INFECTION: The rabbit is placed on its back on the fluoroscope table without its head being fixed so that the bronchial catheter can function properly. After the rabbit is tracheotomized and anesthetized with ether, the catheter is introduced through the opening in the trachea under fluoroscopy. Then the bacillary emulsion is infused into the pulmonary lobe always under the control of the direction-indexwing (w) so as to keep the emulsion flowing into the same side of the rabbit as was previously selected. As soon as the end of the catheter reaches the hilus of the lung, only the vinyl tube, previously loosened and settled in the catheter, is advanced further and further into the chosen lung field without any resistance. Since this tube has already been filled with the bacillary suspension in radiopaque solution, the vinyl tube is seen as a linear shadow moving in a certain direction from the shadow of the end of the catheter. When the shadow of the end of (v) has reached the expected lung field by the adequate mobilization of (v) in the expected direction, the prepared bacillary suspension is immediately injected. A small round homogenous and dense shadow develops. When it seems to be about 0.5 cm in diameter, the quantity of infused bacillary suspension measures about 0.25 cc. This volume corresponds approximately to the quantity of tubercle bacilli required for cavity formation, as described previously.

After the vinyl tube is drawn back into the catheter, the latter is drawn out

with the former and the wound of operation is sutured.

TABLE I. EXPERIMENTAL GROUPS

Group	Onset of Experiment (Body Weight 1.5 kg)	Repeated Injection of Fat Emulsion during 1 Month Preoperatively	Operation Performed on Rabbits to Inducce Tuberculous Infection	Repeated Injection of Fat Emulsion during 6~8 Months Postoperatively	Autopsy
K(F)		$K(F)_6$ +	$K(F)_6$ +	$K(F)_6$ +	
		$K(F)_8$ +	$K(F)_8$ +	$K(F)_8$ +	
K(O)		$K(O)_6$ −	$K(O)_6$ +	$K(O)_6$ −	
		$K(O)_8$ −	$K(O)_8$ +	$K(O)_8$ −	
G(F)		$G(F)_6$ +	$G(F)_6$ −	$G(F)_6$ +	
		$G(F)_8$ +	$G(F)_8$ −	$G(F)_8$ +	
G(O)		$G(O)_6$ −	$G(O)_6$ −	$G(O)_6$ −	
		$G(O)_8$ −	$G(O)_8$ −	$G(O)_6$ −	

Key;
K;……rabbits with induced tuberculous cavity.
G;……normal rabbits.
F;……rabbits injected with fat emulsion.
O;……rabbits not injected with fat emulsion.
6 or 8;……number of months of postoperative observation.

7) SACRIFICING METHOD: In view of the results of ASADA's experiments, fatty substances are probably not deposited much in tuberculous tissue or in the body, following the daily injetion of such a quantity of fat emulsion as mentioned above, which is oxidized and utilized very easily in vivo. So periodical observations after the injection of fat emulsion were necessary in order to tracc the appearance of penetration and distribution of the fat particles into tuberculous tissue.

Rabbits in the K(F)-group were killed by air emboli at varying intervals; immediately after injection, 45 minutes, 1, 3, 6, 12, 24, and 48 hours after the last injection of fat emulsion. All control animals, groups K(O), G(F), and G(O), were sacrificed in the same way, 24 hours after the last feeding.

8) STAINING PROCEDURES OF SLICED HISTOLOGICAL SPECIMENS: Hematoxylin eosin stain (H-E stain), BIELSCHOWSKY-MARESCH (OKA's Modification) silver method (Ag-stain), WEIGERT's rosorcin-fuchsin stain (E-stain), VAN GIESON's connective tissue stain (V. G.-stain), aniline fuchsin stain for tubercle bacilli modified by KUMABE, anilne fuchsin gram stain for tubercle bacilli modified by KUMABE, GOLDMANN's method (SIII-stain) for staining tissue fat, KOSSA's Test for calcium and hematoxylin stain for it, which corresponds to GOLDMANN's method or H-E stain in this experiment, were used.

9) DETERMINATION OF ADEQUATE QUANTITY OF INFUSED TUBERCLE BACILLI: First, because the tuberculous cavity in the chosen lung-lobe must be quite accurately and formed as early and definitely as possible, the writer tried to clarify the relationship between the quantity of inoculated tuberculous bacilli and the period between infection and cavity formation. The diagnosis of

cavity formation was made by periodic chest roentgenograms and confirmed by autopsy.

TABLE 2. RELATIONSHIP BETWEEN QUANTITY OF INFUSED TUBERCLE BACILLI AND NUMBER OF DAYS FOR IDENTIFICATICATION OF CAVITY ON X-RAY EXAMINATION

Rabbit No.	Quantity of Infused Tubercle Bacilli (mg)	Time Required for Initial Identification of Cavity by X-Ray Examination Months Days	Identification of Cavity by Autopsy	Time between Inoculation and Autopsy Months Days	Key; ( )…Uncertain Identification of Cavity †……Dead Case (Diarrhea) Gc……Giant Cavity Note
5	1.0	(0 – 24)	–	0 – 25 †	Cavity Formation occurred almost always 2½ to 3 Months after Inoculation of 1.0 to 2.5mg of Tubercle Bacilli.
4	1.0	3 – 05	+	3 – 06	
3	1.5	2 – 16	+	2 – 20	
2	2.0	(1 – 06)	–	2 – 10	
1	2.5	2 – 10	+Gc	3 – 00	
7	5.0	0 – 21	–	0 – 28 †	Almost no Cavity Formation seen until nearly 2 Months after Inoculation of 5.0 to 7.0 mg of Tubercle Bacilli.
8	5.0	1 – 07	+	1 – 28	
9	5.5	1 – 20	–	1 – 29	
10	5.6	(1 – 00)	–	1 – 05 †	
19	7.0	1 – 26	–	1 – 27 †	
6	10.0	(0 – 19)	–	0 – 25 †	Cavity Formation seen within 1½ Months after Inoculation of 10.0 mg of Tubercle Bacilli.
11	10.0	(0 – 18)	–	0 – 20 †	
12	10.0	1 – 10	+	1 – 18	
14	10.0	1 – 18	+Gc	1 – 19	
20	12.0	1 – 00	+	1 – 00	Cavity Formation seen about 1 Month after Inoculation of 12.0 to 20.0 mg of Tubercle Bacilli.
15	15.0	(0 – 14)	–	0 – 14 †	
21	18.0	1 – 06	+	1 – 06	
18	19.0	1 – 02	+	1 – 28	
17	20.0	1 – 00	+	1 – 00	

From the results summarized in Table 2, in genral, the larger the quantity of tubercle bacilli injected, the earlier a cavity is formed. About I month after inoculation with 12～20 mg of tubercle bacilli (H37 Rv), cavities were almost certainly formed in lungs of rabbits weighing about 2 kg at the beginning of this preliminary experiment, and at this time the several requirements for cavity formation mentioned above, seemed to have been attained in the character and behaviour of these cavities. So the writer used 10～18 mg of tubercle bacilli (H37 Rv) in the rabbits in the main experiments.

III RESULTS (I)……(NON-HISTOLOGICAL FINDINGS)

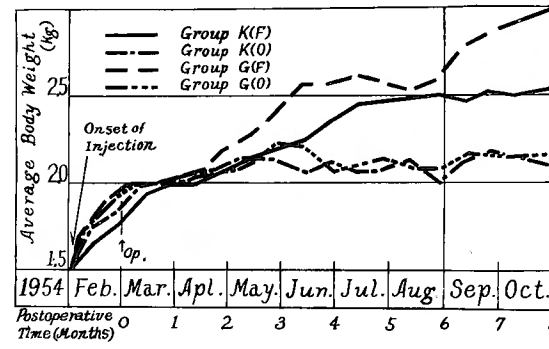
(I) GENERAL SYMPTOMS AND COURSE: Commonly the rabbits in both groups K(F) and K(O), were slow and passive in all their motions and showed a marked loss of appetite for a week after the inoculation; their fur was ruffled and they appeared very lethargic. Following the first postoperative week, such

general changes tended to disappear gradually, and after 10th postoperative day, they returned to almost normal. About 1 month after the operation, however, similar symptoms reappeared to some extent and continued for another month. Afterwards, except for the rainy season and midsummer, K(F)-rabbits became much more energetic and had much better appetites than K(O)-rabbits for 4~6 months until their autopsies. Especially in the above mentioned bad seasons, the rabbits in groups K(F) and K(O), particularly the latter, showed some loss of appetite and inactivity of all motions. The G(F)-rabbits were the most energetic throughout the whole survival period. After the 5th month, even G(O)-rabbtis were slower in all their motions than K(F)-rabbits.

Thus, it can be definitely said that the energy of the rabbits decreased gradually in the following order: G(F), K(F), G(O), and K(O).

2) CHANGES OF BODY WEIGHT: Fig. 2 shows the changes of the average body weight of rabbits in each group. At the beginning of this experiment the rabbits weighed approximately 1.5 kg, but 1~1.5 month later, all of them increased to about 2.0 kg. For the next 1 month or so, their body weights remained the same. Starting about 2.5 months from beginning of this experiment, weight increased in all groups, especially group G(F). The last part of the 4th month and the first part of the 5th month after inoculation corresponded with the so-called rainy season. Until it ended, body weight in the G(F)-rabbits remained stationary, but a gradual increase occurred in the K(F)-rabbits and no change or a tendency towards weight reduction was present in G(O)- and K(O)-rabbits.

Fig. 2.



Variation in average body weight in experimental and eontrolled rabbits. In this experiment, a month was counted as 30 days. (Op.)·· Operation to induce tuberculous infection in rabbits.

From the beginning of July, 1954, no significant decrease occurred in the body weight of any of the rabbits. In August, midsummer, the same tendencies that occurred in the rainy season recurred in all groups. In the period from early autumn to mid-autumn, the rabbits of all groups began to develop more or less increase in body weight, especially G(F)-rabbits, and finally by the time of autopsy (the end of the 8th month after inoculation), the mean body weight reached approximately 3.0 kg in the G(F)-group, about 2.5 kg in the K(F), -G(O)- and K(O)-groups.

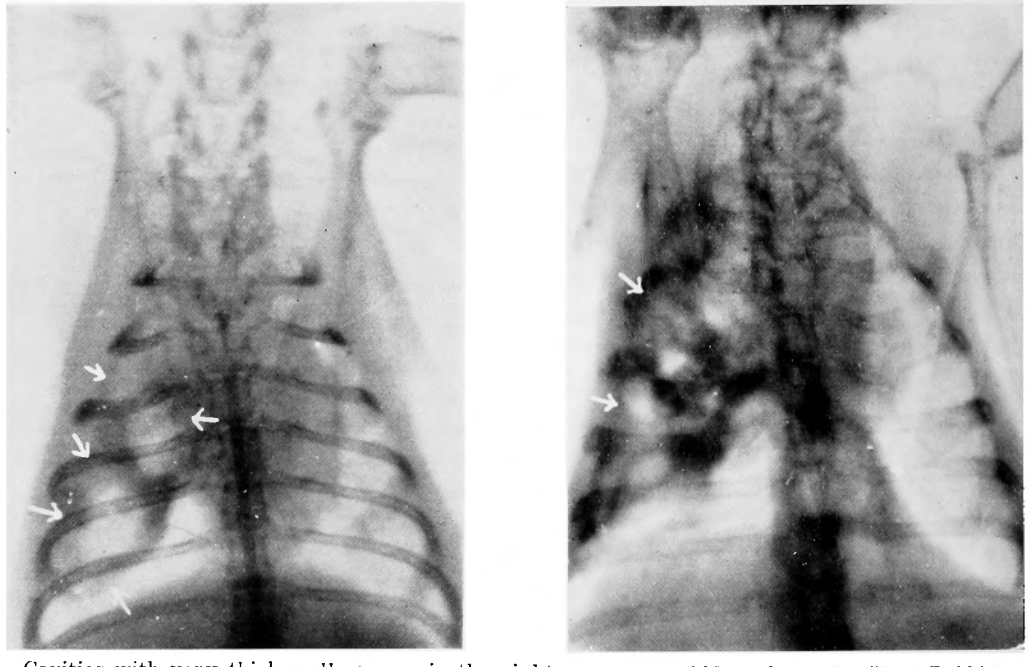
These resuls show that the normal rabbits and even those with pulmonary tuberculous cavities which are as limited in extent as possible, gain weight more rapidly with long-continued administration of fat emulsion than those receiving no

fat emulsion. It is very significant that even rabbits with cavities are able to gain weight better when injected with fat emulsion than the control rabbits receiving no fat emulsion. Another important finding is that even in unhealthful weather which especially causes loss of appetite such as the rainy season and midsummer, there is no loss of weight in the rabbits injected with fat emulsion.

This may be due to a combination of the following four characteristics of the emulsion, which have been demonstrated by others in our laboratory: (1) the combustibility of the fat emulsion itself in vivo, (2) the indirect action of saving body protein, (3) the conditional action of neutralizing against tuberculotoxin, and (4) the direct action of controlling the growth of the tubercle bacilli.

3) FINDINGS IN CHEST ROENTGENOGRAM:

Fig. 3 a. **Fig. 3 b.**



Cavities with very thick walls (→) in the right, upper or middle lobes. (R.…Used Rabbit)
a; R. 65, K(F)8. **b**; R. 63, K(F)6. (Compare with Fig. 4.)

A) K(F)-CAVITIES: For example, in rabbits 65 and 63 (Figs. 3a and 3b), the cavities were generally 1.0 cm to 1.5 cm in diameter, ellipsoid and somewhat flattened from the chest wall towards the hilum but in some rabbits there was a large cavity occupying an entire lobe. A few had exceedingly dense, wide "annular shadows" adjacent to each other, whose inner margins were very smooth and sharp. But the majority of them were as dense as rib-shadows.

These findings probably depend not only on the proliferation of the local connective tissue fibers, but also the calcification as will be described later.

In addition, markedly emphysematous changes were seen in the other lobes. Periodic X-ray examinations showed clearly that the cavities became smaller and

denser as the disease progressed.

The cavities of this group definitely tend to develop a markedly clean, shrinking and consolidated appearance.

B) K(O)-CAVITIES: The "annular shadows", as shown in Figs. 4a and 4b (rabbits 60 and 59), could be traced with difficulty as very uncertain and fine lines. Many of them, mainly those more than 2.0 cm to 2.5 cm in diameter, were larger the K(F)-cavities. The inner surfaces of these cavities showed more uneven-

Fig. 4 a.

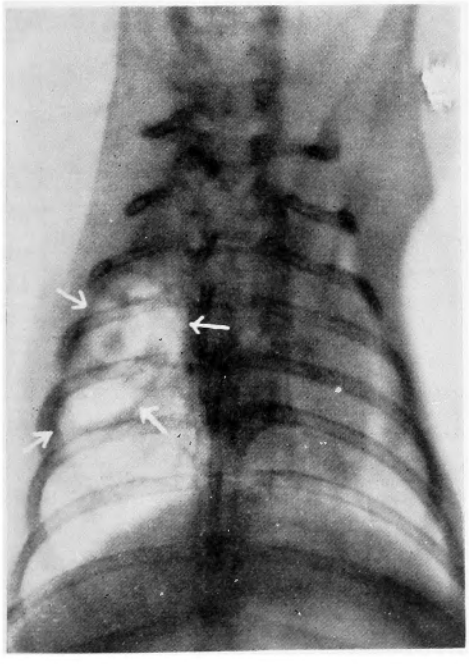
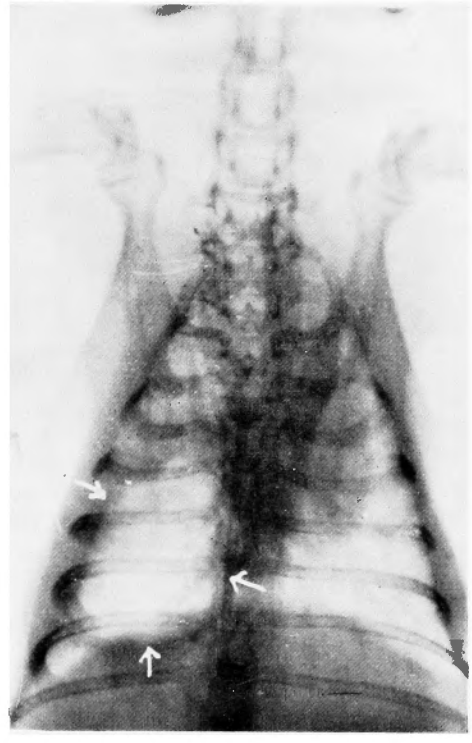


Fig. 4 b.



Cavities with very thin walls (⟶) in the right, upper or middle lobes.
a; R. 60, K(O)8 **b**; R. 59, K(O)6. (Compare with Fig. 3.)

ness and irregularity than those of the K(F)-cavities. No cavities in this group or in the K(F)-group had fluid levels. The cavities seemed to have been insufficiently cleaned and to have fused together. Furthermore, they had the appearance of "tension cavities" or "elastic cavities".

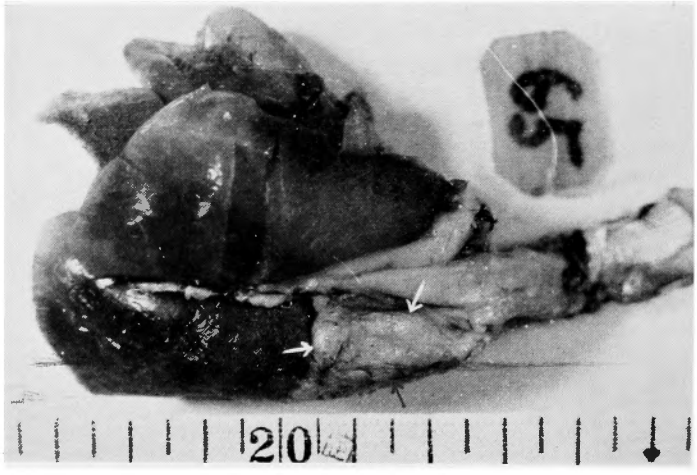
The findings described above in A) and B), indicate that the cavities may be cleaned and their healing tendencies enhanced by repeated injections of this fat emulsion over a long period.

4) GROSS APPEARANCE of the REMOVED LUNGS: In groups K(F) and K(O), the lesions were usually in one lobe and even if there were metastatic foci in other lobes, there were only a few miliary tubercles at most. Even if, sometimes, they had fused together extensively, at least one lobe remained almost

always in nearly a normal condition. No pleural exudate was noted in any of the rabbits.

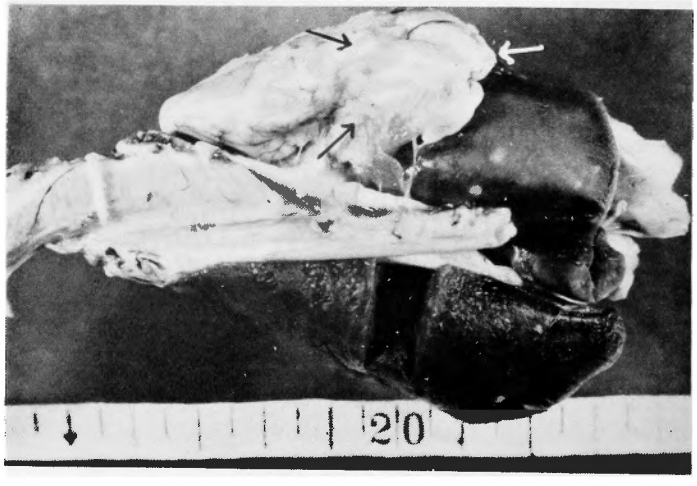
A) K(F)-CAVITES : Rabbit 65 had fibrous, membranous and stronger adhesions between the parietal and visceral pleura extending approximately the length of the main lesion in the lung, which was not as swollen as the other normal parts. On the surface of the main lesion there are many nodular projections whose borders are in contact and sharply demarcated with ditch-like excavations between them. Extending from the excavation, were many small vessels showing extensive proliferation and tortuosity. Very fine branchings from them were found on the surface of each nodule. The consistency of these nodules was mainly elastic and hard, but in parts elastic and soft (Fig. 5). The cut-surface of the nodule had the appearance of a hornets' nest with many chambers separated from each other by fibrous tissue septa. The smaller chambers were entirely filled with caseous debris, except for the center, while the larger ones had already discharged their caseous material. The latter therefore were obvious cavities with fairly smooth inner surfaces. The caseous debris which was grayish white to grayish yellow, was relatively hard and friable, remaining on the

Fig. 5.



Gross appearance of removed lung. The tuberculous lesions occupies a great majority of the right upper lobe alone. Cavity wall (—→) is not so prominent. Dorsal view, R. 65, K(F)8.

Fig. 6.



Gross appearance of removed lung. The tuberculous lesion occupies nearly the whole right upper lobe, cavity wall is very prominent (— →). A few miliary tubercles are found in the right lower lobe alone. Dorsal view, R. 60, K(O)8.

cavity wall to about 0.3 cm in width.

B) K(O)-CAVITIES: Fig. 6 (rabbit 60) shows that the diseased portions of the lungs are generally much more swollen than those of K(F)-group. The surface is generally smooth, showing very minimal nodular swelling, ditch-like formations or tortuosity of small vessels. Varying consistencies, elastic hard, elastic soft and soft, were found in the whole lesions. In these cavities, the consistency was always softer than in K(F)-cavities. The cut surface showed a few cavities, approximately 2.5 cm in diameter, without the hornets' nest appearance, which were filled with caseous debris of mud- and slime-like consistency and grayish white, slghtly yellowish in color. But only the central part of the caseous mass had been discharged. The cavity wall, too, was less well formed than in group K(F).

In view of these facts, it may be said that repeated administrations of fat emulsion may be effective in shrinking and hardening cavities and also in cleaning up caseous debris.

5) X-RAY FINDINGS OF THE REMOVED LUNG:

A) K(F)-CAVITIES: Typical of this group is rabbit 65 (Fig. 7a), in which the cavities are seen as "annular shadows", relatively homogeneous, moderately

Fig. 7 a.

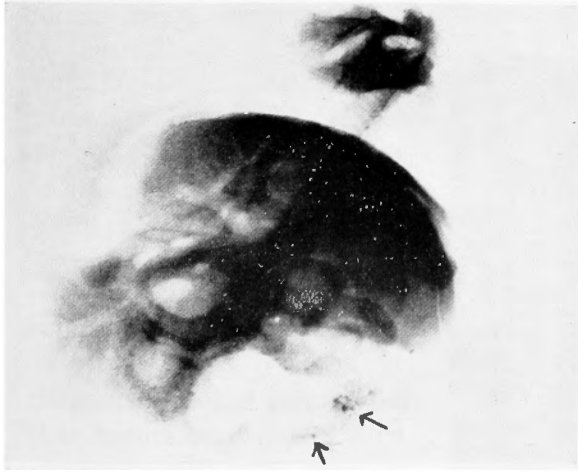
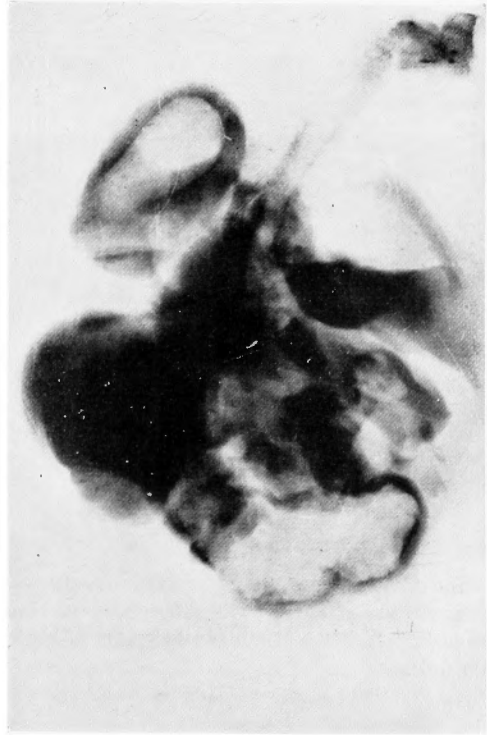


Fig. 7 b.



Roentgenograms of removed lungs. showing presence or absence of calcification of cavities. **a**; R. 58, K(F)6, and **b**; R. 59, K(O)6. Calcification (⟶) has occurred in the former, but not yet in the latter.

dense and wide, and with comparatively well-defined inner and outer borders. Near the inner margin of the "annular shadow", there can be seen very fine dot-like or miliary sized, homogeneous, very sharply bordered and extremely dense shadows, which are as dense as rib shadows, in several places isolatedly or crowded together in one place.

These dot-like shadows themselves may be the beginnings of so-called calcification in cavities.

B) K(O)-CAVITIES: As contrasted with K(F)-cavities, no observations suggesting calcification were seen in these cavities (Fig. 7b).

The findings mentioned above justify the assumption that following repeated injections of fat emulsion, calcification occurs in sites of caseous debris near the inner margins of cavity walls. Up to the present, calcification in cavities has generally been believed to show some degree of healing. The writer, also, wishes to support this belief.

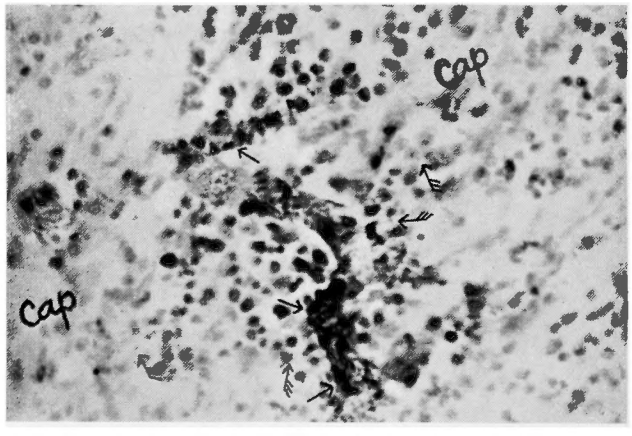
IV RESULTS (II)……(HISTOLOGICAL FINDINGS)*

I) BEHAVIOUR OF BLOOD VESSELS IN CAVITY WALLS.

A) IN RESPECT TO THE PENETRATION AND DISTRIBUTION OF INFUSED FAT EMULSION IN CAVITIES: In group K(F), collections of monocyte-like cells were often found surrounding the blood vessels of cavity walls. Under high magnification, neutral fat granules appeared to be scattered about in the extravascular tissue space of these areas (Fig. 8).

Papillary projections of connective tissue with numbers of small vessels, extending from the inner margin of cavity walls towards the caseous material, were often found. There were usually conglomerates of vessels with monocyte-like cellular infiltration at the base of the projection. As observation proceeded from the base to the end of a projection, the elastic fibers of the blood vessel walls suddenly began to lose stainability with E-stain, or stained only as very pale fine fragments. The Red Blood Cells inside these destroyed vessels also lost ability to be stained by the H-E method, and were transformed into very light yellowish, shining, well-defined, transpa-

Fig. 8.



The fat granules (⟶) existing freely in extravascular tissue space of cavity wall, especially its inside layer, 45 minutes after the combined injection of both particles, fat and carbon.

Note carbon particles adhering to the endothelial cells and penetrating right beneath the perivascular cells(⟶). R. 65, K(F)8, SIII stain, × 400.

* Letters in the figures indicate; B… "BALKEN", C… Caseous Area, Cap… Cavity wall, especially Encapsulation.

rent substances. Eventually, these blood vessel walls themselves and their contents, as a whole, underwent necrosis, or rather necrobiosis.

These projections may have originated in the same manner as the so-called "Kavernenbalken", "Kavernenleisten", SIEGEN's "Spur" viz. "Blutlymphgefaesss tiel", "trabecula", "band", etc.; they will be described below as "BALKENS".

In general, the K(F)-"BALKEN" seemed to be especially thin and long, the K(O)-"BALKEN" thick and short. In the K(F)-group, as shown in Figs. 9 a and 9b, a definite localized and characteristic field (SIII-field), stained orange red with Sudan III, was found adjacent to the tip of the "BALKEN" in the caseous

Fig. 9 a.

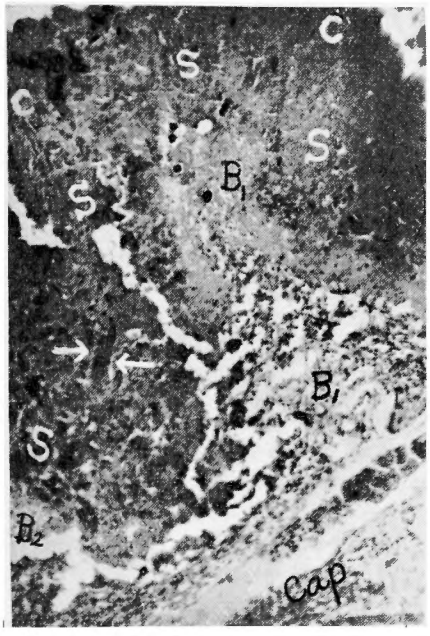


Fig. 9 b.

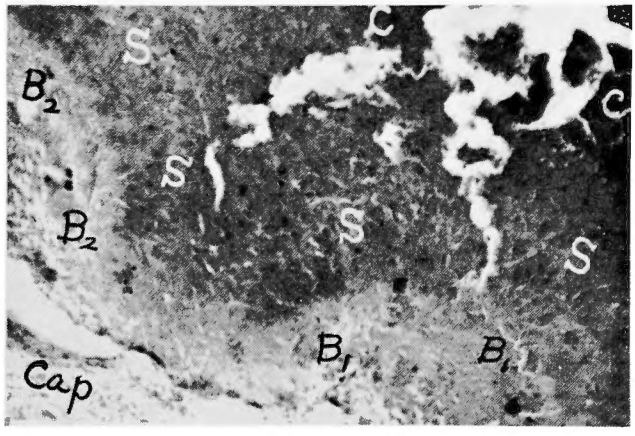
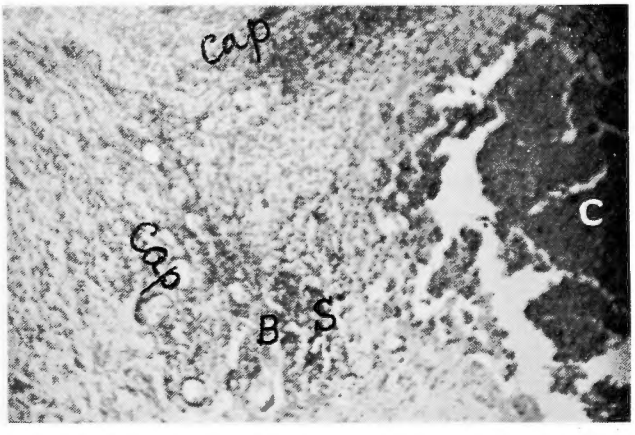


Fig. 9 c.



SIII-fields and "BALKENS" at the following times after the last injection. SIII-stain, × 100.

a; 45 minutes after injection, R. 65, K(F). The SIII-field (S) looks quite black, grossly granular. Note the calcified blood vessels (⟶), and two "BALKENS", B_1 a typical and B_2 an atypical viz. an elevation.

b; 3 hours after injection, R. 69, K(F). The SIII-field (S) looks greyish black. Note two "BALKENS" (B_1, B_2) curved in the excessive part beyond a limit····cf. this text (P. 630).

c; 45 minutes after the injection of carbon suspension only following 24 hour's starvation of controlled rabbit 60, K(O)8. Note the presence of "BALKEN" traceable with difficulty from the site of SIII-field appearing very slightly (S).

material. Under higher magnification, the SIII-field is seen to consist of both dot-like, red granules and red-yellowish stained intermediate substances. Approaching the central zone of the caseous layer from the peripheral, the orang-red granules tend to fade away and gradually become arranged in columns, making a striped pattern with connections between stripes here and there.

In regard to the behaviour of the SIII-fields at varying intervals since the last injection of fat emulsion, there was a peak in the extent, tone and density of the orange-red colour, extending over the period between the 45 th minute and the 3rd hour. The SIII-field, thereafter, became narrower in extent, less dense, and changed from red or reddish orange to orange-yellowish or yellowish in tone. Finally, even 24 hours or 48 hours later, the SIII-field could be represented with SIII-stain. The characteristics after 48 hours, however, were somewhat irregular even in group K(O) (e. g., rabbit 60, Fig. 9c).

Hence, they did not show a significant difference between groups K(F) and K(O). But the appearance of the neutral fat granules, viz. the SIII-field at the period of the peak mentioned above, could definitely indicate a state of equilibrium of time with the previously injected fat emulsion.

B) FINDINGS IN CAVITY WALLS FOLLOWING INTRAVENOUS INJECTION OF FINELY DIVIDED CARBON PARTICLES: In rabbit 65 in group K(F), which was sacrificed 45 minutes after the simultaneous injection of both fat emulsion and carbon particles, several vessels penetrate into the central part of the SIII-field from the cavity wall; they were almost entirely filled with or at least lined with carbon particles.

Here and there, moreover. the carbon particles have invaded right under the perivascular cells (Figs. 10a, 10b, and 10c). Similar findings were observed even at the site of monocyte-like cellular infiltration adjacent the caivity capsule (Fig. 8).

In the K(O)-group, e. g. rabbit 60 (Fg. 10d), the blood vessels of the "BALKEN" were similar in appearance to those in group K(F). In both groups K(F) and K(O), a very small number of wandering histiocyte-like cells, containing innumerable carbon particles in their cell bodies, were rarely found, e. g. rabbit 60 (Fig. 11).

Very few carbon particles were present within the vessels of the inner cavity walls, between two "BALKENS", but they were prominent at the site of the "BALKEN" itself. In these areas, almost no monocyte-like cellular infiltrations were found.

C) RECAPITULATION: It seemed that the anatomical structure of the vessels of the inner cavity walls, especially of the "BALKEN", were already destroyed and denatured as indicated by the findings in preparations treated with H-E stain, E-stain, and Ag-stain (as may be seen below).

The fact that the intravenously injected carbon particles reached the destroyed vessels of the inner cavity walls, may be explained as follows;

Even if the blood within destroyed blood vessels does not retain its normal condition, it at least remains liquid and the damaged vessels themselves still act

Fig. 10 a.

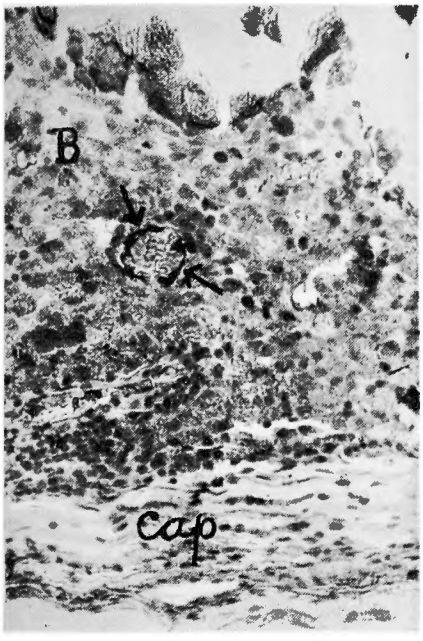


Fig. 10 b.

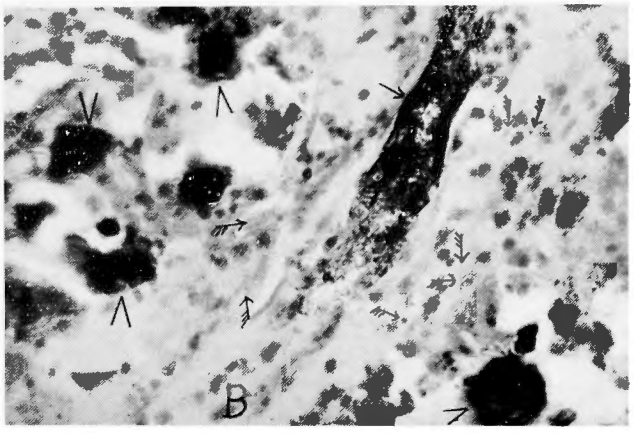


Fig. 10 c.

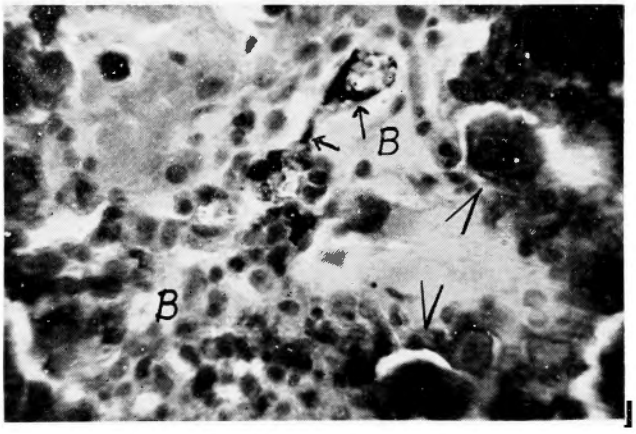
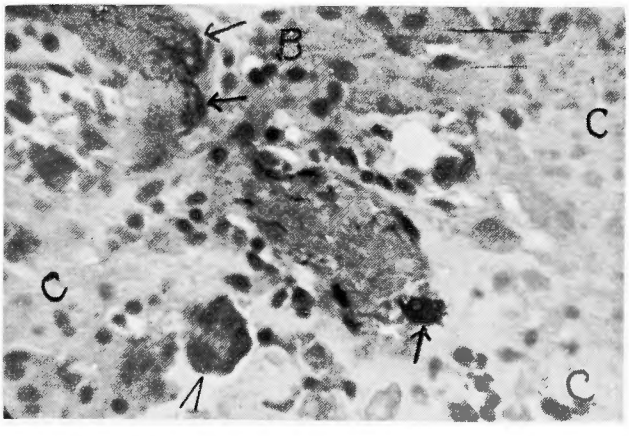


Fig. 10 d.



The diseased blood vessels of special character in "BALKEN" 45 minutes after the combined injection of both particles, fat and carbon. Carbon particles have reach them (⟶). Calcification (>) was found here and there in their neighbourhood. R. 65, K(F)8, SIII-stain, × 400.

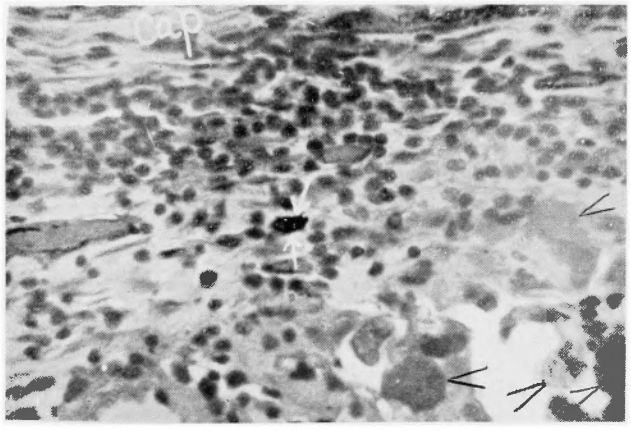
a; Note the stone-wall like arrangement of degenerating Red Blood Cells, made clearer by accumulation of infused carbon particles among them.

b; Note the infused fat granules (⟶) penetrating into extravascular tissue space.

c; Note the carbon particles invading right beneath the perivascular cells.

d; R. 60, K(O)8, for control, was treated intravenously with carbon solution alone. Note the findings similar to above-mentioned a and b.

Fig. 11.



A wandering histiocyte-like cell (—→) containing carbon particles in its cytoplasm. Calcification(>). R. 60, K(O)8, SIII-stain, × 100.

as a part of the blood circulatory system, and since they have not been thrombosed can function as a closed blood flow.

In addition the fact that carbon particles adhere tightly to the endothelium of the vessels and particularly that they penetrate right beneath the perivascular cells, surely suggests a higher increase in permeability of such abnormal vessels. The carbon particles enclosed in wandering histiocyte-like cell, have a similar significance. In regard to the equilibrium in time between the last injection of fat emulsion and the appearance of the SIII-field, there should be no objection to the theory that the previously injected fat emulsion itself can penetrate into the necrotic layer of a tuberculous cavity from the special so-called necrobiotic layer. In this necrobiotic zone, the blood vessels, destroyed from the histological point of view, may still function, and then the SIII-field develops its radiating appearance because of the increasing permeability of the abnormal vessels.

What, however, is the significance of the SIII-field which is already slightly present at the 24th hour or even at the 48th hour after the last injection of fat emulsion?

The well-known "masked fats" in caseous debris are denaturalized on some occasions and then the very "denaturalized fats" may come into the microscopic visual field. If this is so, the possibility that the "denaturalized fats" may be absorbed from the inner margin of the cavity wall, especially the "BALKEN", should be considered.

That is to say, even if no fat emulsion were injected into rabbits with tuberculous cavities, the gathering of these "denaturalized fat" granules might show a radial appearance like that of the SIII-field when fat emulsion is used, and they might be absorbed by the lymphatics of the cavity wall.

This appearance may indeed represent the behaviour of almost constant, unchangeable, durable absorption without regard to the injection of fat emulsion. A part of the fat granules seen in the SIII-field in K(O)-cavities, may be of this kind. On the contrary, of course, it should be kept in mind that the fatty substances in the blood due to tuberculosis (lipemia) may penetrate the necrobiotic area from destroyed vessels under almost constant, unchangeable and durable conditions.

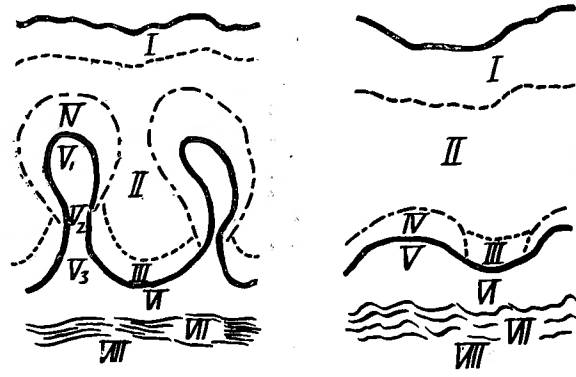
Hence, it may eventually be thought that the great majority of neutral fats in the SIII-field are probably the previously injected fat emulsion itself and a

residual part, the preexisting fat in blood and the "denaturalized fats" included in the caseous debris.

2) BEHAVIOUR OF TUBERCLE BACILLI IN CAVITY: Tubercle bacilli were arbitrarily classified according to 8 divisions of cavity as shown in Fig. 12 and the results are summarized in Fig. 13.

A) K(F)6-CAVITIES: Under low magnification only rod-forms were found in the caseous debris, as if they had avoided divisions IV and V. As observation proceeded from the inner surface to deeper layers of a cavity, in general, an abrupt decrease of the number of tubercle bacilli was seen. In division I, they were too numerous to be counted. Division II showed them in moderate number, while in division III one or two could be found only with great difficulty.

Fig. 12.



The eight divisions of a cavity where tubercle bacilli are distributed. (D.·····Division)

D. I·····Inner surface of cavity and its adjacent area where tubercle bacilli often make a great majority of colonies.

D. II ··· Most of the caseous area except for D. I, III, and IV.

D. III··· The area corresponding approximately to the whole necrobiotic area.

D. IV ··· The area corresponding approximately to the maximal extent of the SIII-field.

D. V ··· The area corresponding approximately to the "BALKEN" itself, consisting of three parts (V_1, V_2, and V_3), namely the end, intermediate and basal parts.

D. VI ··· The area corresponding approximately to the specific encapsulation.

D. VII···The area corresponding approximately to the non-specific encapsulation.

D. VIII···The area outside the non-specific encapsulation.

(left; Cavity when fat is used. right; Cavity when no fat is used.)

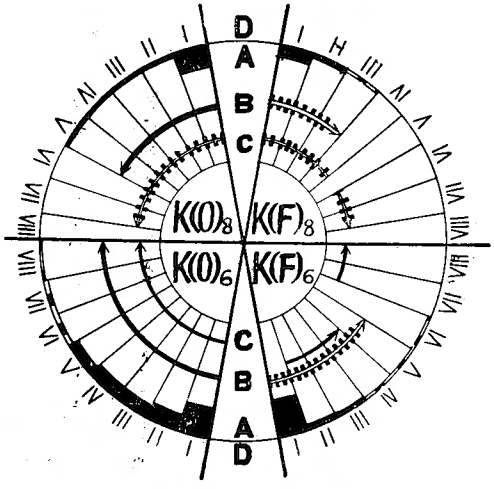
On the other hand, even under high magnification, a very small number of swelling rod-forms were found only in divisions IV and V, and an even smaller number of granular forms of varying sizes, isolated from each other, appeared in divisions IV, V, VI, VII, and VIII.

In regard to the stainability of the two forms, they generally showed non-acid-fastness or slight acid-fastness in all divisions except for divisions V and VI. The nearer they came to division I, the stronger their acid-fastness became. The deeper their division, the more their stainability decreased and the more such abnormal forms as shrinking, swelling, or powdering, etc. appeared.

B) K(O)6-CAVITIES: As compared with K(F)6-cavities, under low magnification, the rod-forms in these cavities were very numerous and comparatively uniform in their distribution, and their concentration resembled that in K(F)6-cavities.

Moreover, the rod-forms were generally very coarse and large, and the granular

Fig. 13.



Changes in characteristics of tubercle bacilli alive in cavity, following injections of fat emulsion.

K(F)8-group upper right and K(F)6-group lower right.

K(O)8-group upper left and K(O)6-group lower left.

A···Distribution of rod forms according to divisions (transversely), and approximately rate of density (vertically).

(Under low magnification)

(Under higher magnification)

B···Distribution of rod forms, with acid-fastness (⇒) and with non-acid-fastness (⟶).

C···Distribution of granular forms, under the high magnification, with acid-fastness and with non-acid-fastness.

D···The Eight Divisions (I~VIII), as shown in Fig. 12.

Note; Observation of their stainability was carried out in the rod or granular forms, mainly with acid-fastness and mainly with non-acid-fastness.

particles included in them were also quite large. A great majority of these two forms were almost completely non-acid-fast and stained easily. The deeper layer, the more their stainability decreased.

C) COMPARISON OF DIFFERENT SUBGROUPS: Both rod-forms and granular bodies in these K(F)8-and K(O)8-cavities tended to be fewer than in the K(F)6-and K(O)6-cavities. There was a tendency, too, that the more deeply into the cavity the observations were carried out, the more numerous the granular bodies became, and simultaneously the intenser the acid-fastness of both types became. All tendencies of these two forms existing in K(F)8-cavities were markedly higher than those of the K(O)6-cavities.

The results indicate that following repeated injections of fat emulsion, the tubercle bacilli in cavities may develop a tendency to be transformed from easily stainable non-acid-fast to hardly stainable acid-fast forms, and, moreover, the rod-forms may be reduced moderately in number. Their distribution division in the cavity becomes narrower, and they are more concentrated in the lower divisions than in the higher. Even if tubercle bacilli are observed in sites where infused fat granules penetrate from the necrobiotic vessels,……SIII-field or division IV……, they have already been transformed into such atypical forms as shrunken, swollen, and destroyed, and are greatly reduced in number.

Therefore, it may be said at least that repeated injections of fat emulsion cause many changes in the characteristics of the tubercle bacilli in cavities. Two actions of the infused fat,···neutralization of tuberculotoxin and inhibition of growth of tubercle bacilli reported by ZAITSU and OTANI in our laboratory···, should, of course, be considered.

Fig. 14 a.

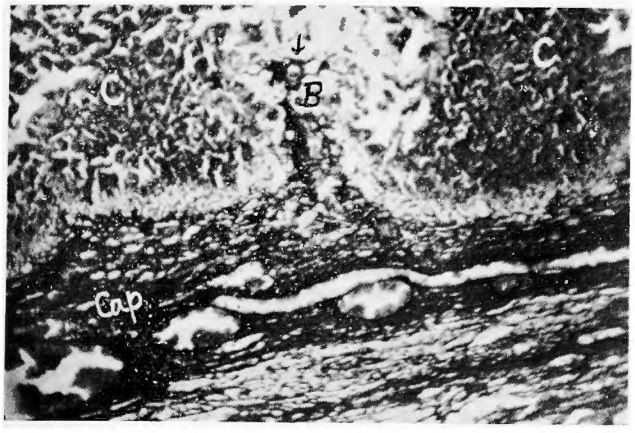


Fig. 14 b.

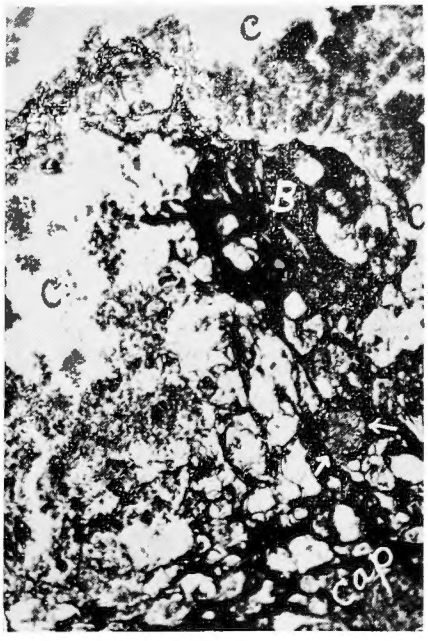


Fig. 14 d.

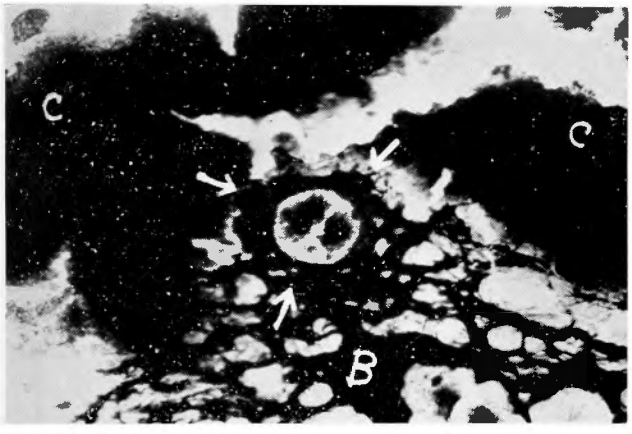


Fig. 14 c.

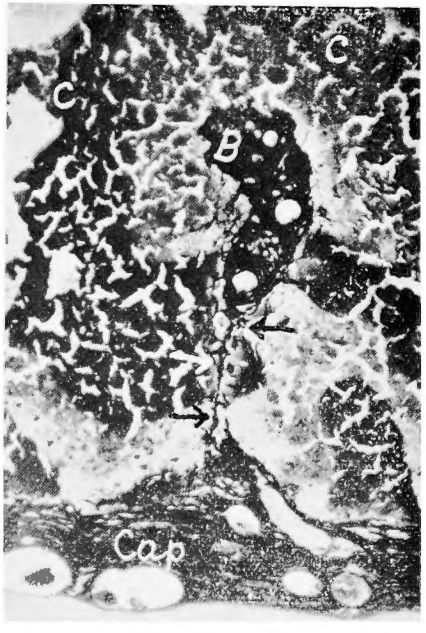
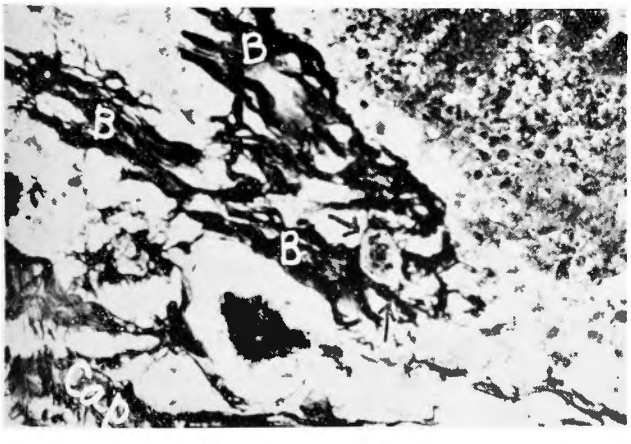


Fig. 14 e.



Various shaped argyrophilic fibrous framework of "BALKEN", often involving irregular found spaces (⟶) filled more or less with some formed material. Note marked proliferation but little destruction of argyrophilic fibers K(F)-group, Ag-stain.

a; Framework of a "BALKEN". R. 63, K(F)6, × 100.
b; Framework of another "BALKEN" of the same preparation as Fig. 14a, × 400.
c; An end of the framework remaining as an island in the caseous area, which is still connected with the fibrous cavity wall by a few fibers (⟶). R. 65, K(F)8, × 100.
d; A star-shaped arrangement of fibrous mass including a round space filled with some formed material and projecting into the caseous area as a elevation of fibrous encapsulation…a very atypical "BALKEN". R. 62, K(F)8, × 400.
e; Framework of another "BALKEN" of the same preparation as Fig. 14d, × 400. Note its course changing on the way (left upper, right lower, again left upper right).

3) FINDINGS OF CAVITY WALLS USING SILVER STAIN.

A) K(F)-CAVITIES: As my be been in Figs. 14a and 14b (rabbit 63), argyrophilic fibers encircling these cavities, in general, are greatly increased, and each of them is very coarse and large. They are closely parallel and arranged quite regularly. They have also been transformed into collagenous fibers.

In one part of a cavity wall, argyrophilic fibers arranged in parallel showed as a slight elevation extending somewhat towards the inside of the cavity wall. The nearer the top of the elevation, the coarser were the argyrophilic fibers, and then they coincided with the argyrophilic fibers of the ends of the "BALKEN", as will be described later.

In other parts, the argyrophilic fibers which were on the inner margin of the cavity wall and were just in contact with the caseous debris, were often broken into fragments and slightly out of order. Part of their destroyed pieces were sometimes vertical or sometimes oblique to the cavity wall.

Subsequently under low magnification, about one or two conglomerates of argyrophilic fibers per visual field extended into the caseous debris vertically from the cavity wall consisting of plentiful coarse, highly collagenized fibers. In comparing these with the findings of the same portion of each preparation stained by other methods, these conglomerates seemed to be the fibrous skeletons of the "BALKEN".

As observation was carried out from the base of a "BALKEN" towards its end, the proliferation of the argyeophilic fibers and their transformation into collagenous fibers increased, so that there were almost no spaces visible between them.

In the intermediate and basal parts of the "BALKEN", these fibers sometimes ran separately in various directions……vertically, parallel, or oblique…against the fibrous encapsulation of the cavity, so that the fibrous conglomerates made a very shaggy frame-work. However, sometimes these fibers were very finely broken and at last their disordered fragments disappeared. Consequently, the ends of the "BALKEN", broken away from their bases, were seen separated in the caseous zone as islands (rabbit 65, Fig. 14c). In the centers of these 'islands', some irregular round, vacant spaces were often found. Other irregular round spaces existed also in the centers of star-shaped, radially arranged fibrous masses among the numerous skeleton fibers running in various directions. (Figs. 14d and 14e, rabbit 62).

These spaces may be thought of as blood vessels, when compared with the findings in H-E, SIII, and E-stain preparations etc.

These facts indicate that fibrous proliferation in cavity walls of K(F)-group is very marked. However, invasion by tubercle bacilli and breaking of the fibrous

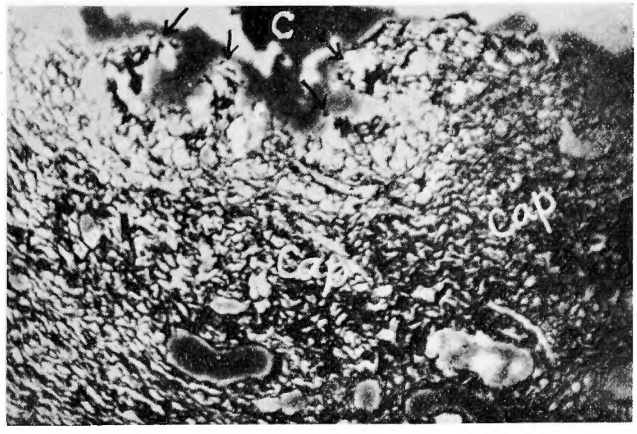
encapsulation of cavities are almost always found to some degree.

B) K(O)-CAVITIES:

When compared with the argyrophilic fibers of K(F)-cavities, for example rabbit 59(Fig. 15), the quantity of the fibrous mass of these K(O)-cavities was generally small.

The fibrous encapsulation of cavities being very fine, there was a sparse and wavy arrangement, and the spaces between the fibers were large. As the observations extended to the inner margin of the fibrous capsule from its outer layer, the fibers were seen to produce a framework, and they were apt to change their directions to become vertical to the cavity wall, finally extending towards the caseous debris.

Fig. 15.



Poor growth of argyrophilic fibers in cavity encapsulation and "BALKEN" itself. Note the wavy and finely meshed arrangement of fibers in the cavity wall, and little proliferation but eminent destruction of argyrophilic fibers in the inner layer of fibrous encapsulation, especially at the bases of four "BALKENS" (⟶). R. 59, K(O)6, Ag-stain, × 100.

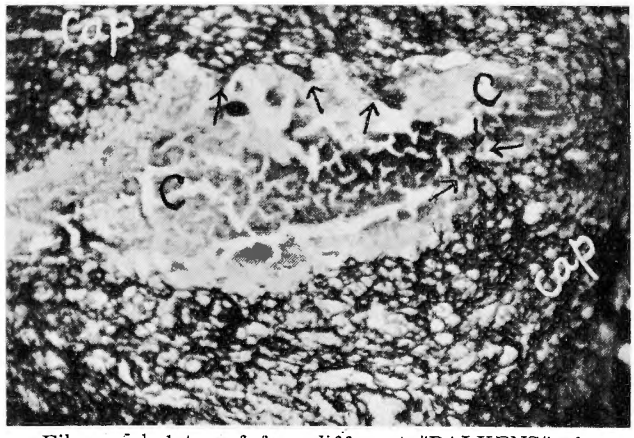
As for the fibrous skeleton of the "BALKEN", which were generally more atypical in these cavities than in the K(F)-cavities, the base was very wide, the height very low so that many of these fibrous masses were observed only as a elevation of the inner margin of the fibrous encapsulation. But all the tendencies observed in K(F)-cavities, were present, more or less, in these K(O)-cavities.

Hence, the productive changes in the fibers in these K(O)-cavities were smaller, and the degree to which the fibrous encapsulation was broken and invaded was more extensive and deeper reaching than in the K(F)-cavities.

C) COMPARISON OF DIFFERENT SUBGROUPS:

In comparison with K(F)6 cavities, K(F)8-cavities were surrounded with much thicker, denser and relatively straight fibers, and also the width of the capsule as a whole was

Fig. 16.



Fibrous skeleton of four different "BALKENS" of poor growth (⟶). R. 54, K(O)8, Ag-stain, × 100. (compare with Fig. 15.)

rather narrower. All these fibers were stained clearer red by V. G.-stain.

Furthermore, the K(O)-cavities (rabbit 54, Fig. 16), generally looked slightly productive and the fibers in the fibrous capsule paralleled each other, but they were still more wavy so that the width of the capsule, as a whole, was thicker than that of K(O)6-cavities.

Also the fibrous capsule of the K(F)8-cavities tended to appear more productive and shrinking than that of the K(O)8-cavities, and their transformation into collagenous fibers was apt to occur earlier.

The above-mentioned findings indicate that following infusion of fat emulsion, even though such features as the tubercle bacilli themselves, their metabolic products and tissue debris etc. invading and breaking down the cavity wall were found slightly here and there, the argyrophilic fibers almost always showed marked proliferation and their transformation into collagenous fibers was accelerated.

Thus the longer the administration of the fat emulsion is continued, the greater are the above mentioned tendencies.

4) CALCIFICATION INTO CAVITY: There have been no histochemical reactions by which calcium alone in tissues can be definitely identified. However, any substance having simultaneously at least the three characteristics listed below, may be considered to be calcium itself without any objection, even though each characteristic by itself does not always identify calcium.

1.) It must stain blue or blue violet with Hematoxylin i. e. GOLDMANN's method or H-E stain, 2.) it stains black with KOSSA's silver method, and 3.) it shows dot-like dense shadows in X-rays of removed lungs, as already described.

In this experiment, the substances with any one of these characteristics are very similar in regard to their position in the cavity and to their formation as described below. Thus the writer thinks that calcium in tissue can be identified almost exactly.

(i) FINDINGS USING GOLDMANN'S METHOD:

A) K(F)-CAVITIES: The caseous debris was stained, as a rule, dark blue, blue or blue violet, and stained many fine granules which stained orange red or red with SIII stain as well as many irregularly shaped objects located in the SIII-fields.

Under higher magnification, these objects were somewhat larger than an ascaris-egg and had various shapes; mulberry-like, spherical, elliptical, guitar-like, fertilized-ascaris-egg-like, etc. Their surfaces appeared somewhat coarsely granular, uneven and irregular. The granular formations ranged in size from Red Blood Cell to lymphocyte-sized.

Only a small part of the circumference of these objects was distinctly bordered with inner and outer strands which were stained deep clear blue. A light bright blue transparent zone between these two strands was definitely seen. Thus the border zone seemed to act as a mantle to these objects or as a shell, such as that of a fertilized-ascaris-egg. (Fig. 17a.)

On the other hand, the contents···the central zone of the object···was nearly transparent and structureless. Under comparatively dark visualization, however, they

had the appearance of a stone-wall. That is to say, they formed a conglomerate of spherical substances, of about the size of Red Blood Cells, very light bright yellow, almost transparent, somewhat unevenly outlined (Fig. 17b, rabbit 63). Especially in the boundary zone of the caseous layer, these objects···the mantle and its contents, as a whole,···were plentiful, stained deep red, and crowded together. Towards the inside of the cavity wall in the caseous layer, they decreased markedly in number and were apt to be isolated and stained more lightly.

Subsequently, as was seen in Fig. 9a, various kinds of hyaline formations, stained pale blue or bright blue, long and slender, unpointed at their ends, somewhat curved, and somewhat dumb-bell-shaped, were found, in general, to be confused with each other in the caseous zone. These were arranged in nearly parallel pairs, so that a little space was left between the two members of a pair. This suggested something like a canal-like formation and also sometimes seemed to be like a branching tree, sending out a root from the end of a "BALKEN". In the inner space in this canal-like formation, neutral fat granules stained orange red by SIII-stain were found among the bright colorless objects, which seemed to be like a mass of

Fig. 17 a.

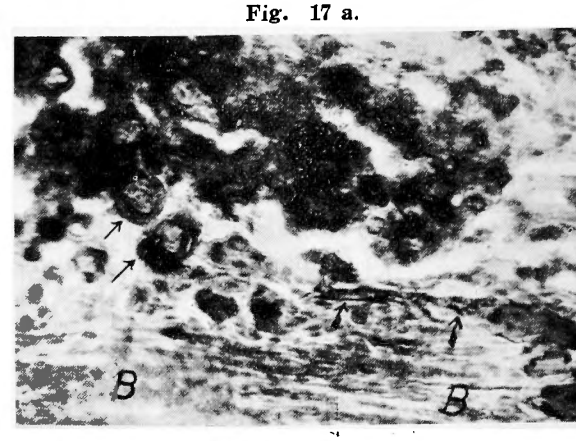


Fig. 17 b.

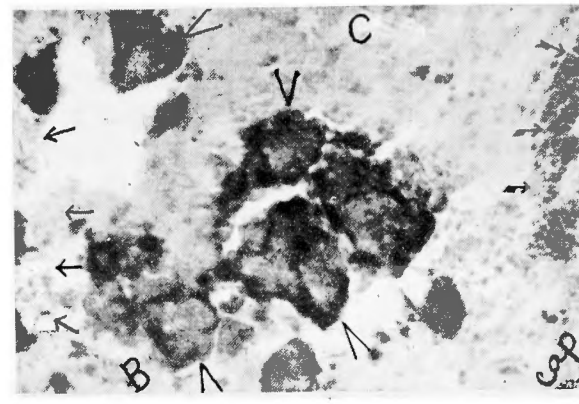
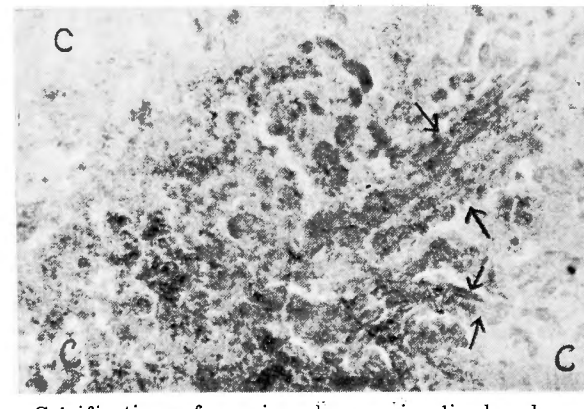


Fig. 17 c.



Calcifications of varying shapes, visualized under comparatively dark condensation. K(F)6, SIII-stain, × 400.

a: Ascaris-egg like objects of calcification (—→) including some formed material resembling a stone-wall and dot-like neutral fat granules (black dots). Mulberry-like objects (upper, look black, granular,) Note carbon particles inside the blood vessels of "BALKEN" (—→) R. 65, K(F)8.

b; Calcium deposits (>) including some formed material under comparatively dark visualization. Note blood vessels, degenerating (⟶) and almost normal (⇝). R. 63, K(F)6.

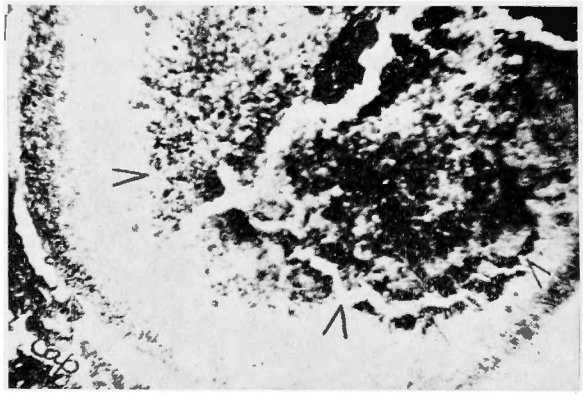
c; Canal-like formations consisting of various kinds of hyaline formations (⟶). R. 63, K(F)6.

degenerating Red Blood Cells and finally looked like a stone-wall. (Fig. 17c)

Since there was a very close resemblance between the contents of this canal-like formation and the objects surrounded by the mantle-like formation described above, and since these findings were similar to those of the vessels themselves and their contents in the necrotic area examined by SIII, H-E, and E-stains, it may be that these two formations represent a vertical or cross section of vessel walls themselves and the contents consist of degenerated blood cells. Moreover, calcification may have developed in the skeleton of the abnormal vessels in the necrobiotic layer. The bright, scarcely stained formations, i. e. the contents of partly or entirely calcified vessels, may be nothing but degenerated Red Blood Cells and the orange red stained granules may be explained as follows:

Before the intravenous injection of carbon particles mentioned above, the previously infused fat particles, which had reached the vessels of the tuberculous lesion just before the occurrence of coagulation necrosis of their contents, might have been involved in the process of thrombosis. This may be the reason why no cases were observed with simultaneous remaining of infused fat and carbon particles in those degenerating (hyalinized) vessels.

Fig. 17 d.



d; Typical central calcification (>) of a daughter tubercle adjoining a main cavity. R. 63, K(F)6, × 100.

Even though the lesion (rabbit 63, Fig. 17d) looked like a daughter tubercle next to a main cavity, and was already surrounded with a thick fibrous encapsulation, it consisted of a large amount of calcium deposit, especially in the central area of the lesion, much more than in the main cavity.

This fact seemed to indicate a definite difference between these two lesions in regard to the in vivo reaction against the invasiveness of the tubercle bacilli at different stages.

B) K(O)-CAVITIES: The caseous debris in these cavities was generally stained much lighter blue by Hematoxylin than that in K(F)-cavities. The various bodies mentioned above were not found at all or only a few in the caseous zone. Although the branch-like or tree-shaped formations could be seen, no granules stainable with SIII were involved in them. Moreover, even if there were daughter tubercles, each of them as a whole was too large to be brought into one visual

field. They may have fused together one by one in the course of their developing so that at last only a main cavity remained.

C) COMPARISON OF DIFFERENT SUBGROUPS: In regard to the various kinds of previously described hyalinized bodies appearing in cavities, K(F)8-cavities contained more than K(F)6-cavities, but there were no significant differences between K(O)8- and K(O)6-cavities.

(ii) FINDINGS USING KOSSA'S SILVER REACTION:

A) K(F)-CAVITIES: Under low magnification, many irregularly shaped, large or small, grossly granular, black stained bodies were present at the site corresponding approximately the SIII-field. Sometimes they were arranged in concentric layers (rabbit 62, Fig. 18a).

Under high magnification, some of them were almost round. Their surfaces were irregular and grossly granular. Their central part was a very light brown and with difficulty a framework like a stone-wall could be seen. What corresponded to each stone measured about the size of a Red Blood Cell under comparative dark visualization. The black stained bodies were more numerous in K(F)8-cavities than in K(F)6-cavities.

B) K(O)-CAVITIES: The black stained bodies mentioned above were not found or were only a few in these cavities. (rabbit 54, Fig. 18b). There were almost no differences between the K(O)8- and K(O)6-cavities, in regard to their deposits.

Fig. 18. *a.*

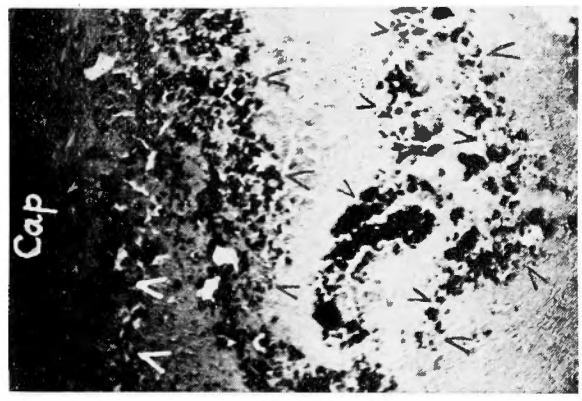
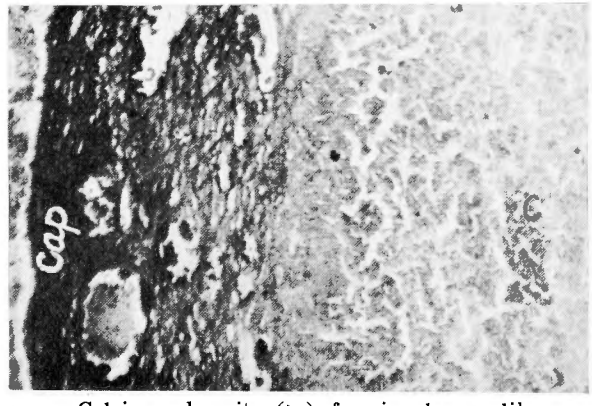


Fig. 18 b.



a. Calcium deposits (>) forming layers like concentric circles in the caseous area. R. 62, K(F)8, Kossa's stain, × 100.

b. Nearly no calcium deposit found in cavity of control rabbit. R. 54, K(O)8, Kossa's stain, × 100.

From the above findings, (i) and (ii), in addition to the already described results of X-ray examination of removed lungs, it may be said indeed that the repeated administrations of fat emulsion are followed by an increasing tendency of calcium to be deposited in cavities, especially in the SIII-fields.

So it seems that calcification is apt to occur in the diseased vessel walls of the

necrobiotic layers in K(F)-cavities and that this tendency parallels the long-duration of repeated infusions of fat emulsion.

However, since even in calcified tuberculous lesions, tubercle bacilli have been found alive, calification alone does not always suggest perfect healing of tuberculous lesions, but it has been considered an indication of the relative healing tendencies of a lesion in the following sense.

It is generally believed that the tubercle bacilli in calcium deposits are at rest and show little, if any, tendency to invade the living body under certain conditions.

Thus, in this experiment, calcification, which was recognized as occurring as the result of daily injections of fat emulsion, may be considered in the same sense.

5) FINDINGS IN LIVER AND LUNG OUTSIDE THE LESION: The liver is well-known to be the most important organ concerned in fat metabolism. When long-continued administration of the fat emulsion has been carried out in rabbits with poor ability to handle fatty substances, especially in young rabbits as indicated by ASADA, if the fat emulsion has not been utilized smoothly enough in its metabolic processes, stagnation of fat might be expected in this organ sooner or later. Also, if there are any toxic action of the fat emulsion on the living body, the liver should represent most directly at least one part of the results due to the side actions. Especially when this type of fat nutrition is studied in rabbits with chronic tuberculosis, which is often apt to cause a diffuse degeneration of the liver, the onset of rapid and severe degeneration might be expected.

Therefore, the writer examined this organ particulary in order to search for possible side actions of the infused fat. A part of the lungs aside from the cavity or lesion was simultaneously examined to determine whether fat embolism had occurred anywhere.

The results indicate that no pathologic findings were found anywhere in these two organs in either the K(F)- or the G(F)-group, such as fatty degeneration, fat embolism, foreign body giant cell formation, inflammatory cell infiltration, granuloma formation, etc..

Neutral fat granules, stained orange red or red with SIII, were, however, contained in both Kupffer cells of the liver and alveolar phagocytes of the lung. They disappeared from the visual fields in this experiment somewhat earlier than in ASADA'S (cod liver oil emulsion used). No tuberculous lesions were found in any of the livers.

Hence, it may be said that even if fat emulsion is injected into a rabbit, diseased or not, for long periods, no pathological tissue reactions occur against the infused fat, nor is any toxic action caused in the living body by the fat, especially foreign body reactions; i.e. no side actions due to infused fat appear anywhere in rabbits.

V DISCUSSION AND SUMMARY

(I) HOW DID THE DAILY INTRAVENOUS INFUSION OF FAT EMULSION, MADE IN OUR LABORATORY, ACT ON EXPERIMENTAL

PULMONARY TUBERCULOSIS OF RABBIT WITH TUBERCULOUS CAVITY AND ALSO ON THE CAVITY ITSELF?

In spite of their pulmonary tuberculous cavities, the experimental rabbits could survive for a long period under intravenous fat nutrition. Despite of some unhealthy climatic conditions, they kept up their daily food intake and their body weight almost always showed a tendency to increase rather than to decrease. Periodical chest roentgenograms and the gross appearance of removed lungs, showed definite shrinking and hardening of the cavities.

It was also shown histochemically that the predominant proliferation of argyrophilic fibers occurred very markedly in the cavity walls and nearly all of them had already changed into collagenous fibers.

Considerable changes in distribution, shape, and staining characteristics of the tubercle bacilli within the cavities were recognized and also calcification in cavities was especially marked at the boundary zone of the caseous layer.

These findings indicate, indeed, a relative healing tendency of the cavity, resulting from the long-continued infusion of fat emulsion.

Although injections of the fat emulsion was injected at least 210 times in each rabbit, no harmful effects due to the fat emulsion could be observed directly or indirectly in either general and histochemical findings.

Thus it is to be understood that, as indicated already by ASADA, most of the infused fat is transformed into the fat depots of the living body, from whence it may be mobilized anywhere when caloric energy is required, and finally it may be entirely oxidized and utilized in tissues.

Therefore, it can be definitely stated that the use of our sesame oil emulsion is at least more effective against tuberculosis than not using it.

(II) HOW DID THE EMULSION PENETRATE INTO AND BECOME DISTRIBUTED OVER THE CAVITIES?

Before MENKIN, many investigators had pointed out the retention at the site of an acutely inflamed area of various substances (e. g. dyes, iron, graphite, foreign proteins, carbon). But he indicated that repeated intravenous injections of a solution of ferric chloride are followed by the deposition of iron in tubercles of the lungs. Using carbon particles instead of the ferric chloride of his experiment, may yield an effect similar to the one he found in tubercles.

MARKHAM & FLOREY undertook to make clear how "micrococcin", a kind of antituberculous biotic made by SU, penetrates into or accumulates in tuberculous foci. They injected "India Ink" intravenously into rabbits with experimental tuberculosis every day or two and observed how the "India Ink" particles penetrated into or accumulated in the lesions. They had assumed that there might be a very close resemblance between "micrococcin" and the carbon particles of "India Ink", as both of these substances, infused intravascularly into tuberculized rabbits, would be distributed over the whole body and the lesions.

The resuts showed that especially since the injection had been done in the early stage of chronic inflammation, the carbon particles (India Ink) were found diffusely

and abundantly in the caseous or central part of the tubercle; however, when injections had started at a later stage, the caseous centers contained little or no carbon and the concentration of carbon increased from the central area to the peripheral, especially epithelioid cells.

At any rate, these observations correspond approximately to the writer's findings at varying intervals after the terminal combined injection of fat emulsion and finely divided carbon particles.

Neverthless there are some differences between MARKHAM & FLOREY's or MENKIN's obsesvations and the writer's, for the two former are lacking in findings at various intervals (minutes to hours) after the last injection.

MARKHAM and his co-worker assumed that after penetration into a tubercle from some capillary, the infused "India Ink" particles were ingested by macrophages and monocytes; these cells containing carbon particles would divide, proliferate, and finally change into the "epithelioid cells".

Originally carbon or "India Ink" particles are chemically and biochemically very inert, stable substances; once entering into the living body, they will have to remain as a foreign body in vivo somewhere until they are discharged.

In this experiment, the increase in permeability of diseased vessels, as reported by YASUHIRA and YAMAMOTO, was proved definitely to be in the necrobiotic layer of the tuberculous cavity, especially at the site of the "BALKEN". Then, after permeation through diseased vessels, the infused fat particles must penetrate the boundary zone of the caseous layer mainly from the site of the "BALKEN" and then evidently represent the "SIII-field" in that place, where cellular elements (e. g. macrophages, monocytes) are almost totally absent. This is the main difference between MARKHAM & FLOREY's observations and the writer's.

In view of the very easy oxidation and utilization of infused fat in normal rabbits, the fat granules penetrating into necrobiotic or caseous areas may be consumed as a source of caloric energy by the tubercle bacilli or the host. This is the fundamental difference between fat and carbon particles···the former takes part in metabolism, but the latter does not.

In regard to the behaviour of these two kinds of particles at relatively early stages after their penetration into an inflamed area from the abnormal vessels, an explanation similar to MARKHAM & FLOREY's opinion (phagocytosis) can be partly made but it is not always so.

The fact that almost no macrophages or monocytes were found in the SIII-fields showing changes in features and characteristics in ratio to the varying duaration after the last injection of fat emulsion, should demonstrate the direct dissemination of infused fat granules to the SIII-fields.

The writer is of MARKHAM & FLOREY's way of thinking in respect to macrophages and monocytes at any rate, because wandering histiocyte-like cells containing many carbon particles and monocyte-like cells containing fat particles were found.

Even in tuberculous rabbits, the great majority of the infused fat particles must be ingested by the cells of the reticuloendothelial system of lung, liver, spleen,

etc. as in normal rabbits as reported by ASADA; but at least a few of them must surely escape into the caseous layer from the diseased vessels in the cavity wall. Moreover, repeated injections of fat emulsion are given, and it is well-known that many fatty substances are originally involved in tuberculous foci, particularly in the caseous central areas, where there are no living cellular elements. Regardless of the writer's assuming the accumulation of fatty substances (infused fat and original tissue fat) in tuberculous lesions, especially in the caseous layer, the caseous areas can not be stained by SIII so diffusely or deeply as had been expected···the infused fat is not stored anywhere in the caseous areas.

This seems to show that these fats (infused fat and denatured tissue fat) in cavities may be finally broken down quite markedly by some mechanisms or absorbed again by the local lymphatic system.

Up to the present it has been generally said that calcium is apt to be deposited where fat previously existed. In this experiment also, since calcification tends to occur near the inside margin of fibrous cavity walls and sometimes shows the arrangement called "Liesegang's ring", fatty substances also, entering the cavity, usually have a similar appearance.

Thus it is true here also that, as is well-known, fat and calcium in tissue are closely related, since an intimate relationship between continuously infused fat and the appearance of calcium in cavities has been marked.

Some degree of analogy should exist between calcification in tuberculous caseous area, it may be broken down into glycerin and long-chain fatty acids by the lipase in that area. The latter may produce a soap with the calcium of the circulating blood.

The presence of alkaline phoshatase in the peripheral zones of tubercles but its absence in the caseous centers was reported by HORII, TAKEUCHI and TAKAMATSU, and by PRINA. According to GROGG and PEARSE, "Alkaline phosphatase usually occurred only in the cytoplasm of polymorphs and in the vascular endothelia within the lesion, but free alkaline phosphatase was present in the necrotic areas. Single group of mononuclear phagocytes within the lesion gave a strong positive reaction for esterase in their cytoplasm". This distribution of alkaline phosphatase in tubercles is very similar to that of both fat and calcium within the cavity in this experiment.

Therefore alkaline phosphatase may liberate orthophosphoric acid from the organic phosphate derived from circulating blood; finally calcium phosphate, chemically and biochemically very stable, may be produced from the above soap and the liberated orthophosphoric acid in the long course of experimental tuberculous disease.

An accurate solution of these problems awaits many future investigations.

(III) WHY DO FATTY SUBSTANCES ACCUMULATE IN THE CASEOUS CENTRAL AREA OF SMALL TUBERCULOUS LESIONS AND IN THE PERIPHERAL ZONE OF LARGER TUBERCULOUS LESIONS? AND ALSO WHY AT TIMES DO THEY APPEAR IN SUCH LESIONS AS CONCENTRIC CIRCLES?

Under low magnification, universally, the height of "BALKEN" seems to have a certain limit. Even if it is too high to be included in one visual field of low magnification, the excessive part beyond the limit is parallel to the fibrous cavity wall (cf. Figs. 9b and 14e). Thus a whole "BALKEN" can be placed in one microscopic visual field. Even when the most extensive SIII-fields occur, they may also be limited to a certain measure (Fig. 19, circle F, marked with oblique lines).

Fig. 19.

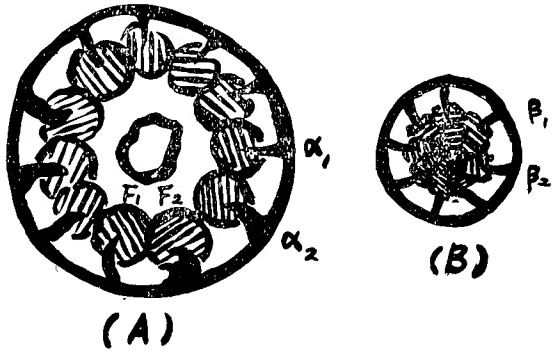


Diagram to illustrate the correlation between the dispersion of infused fat emulsion in a cavity and the size of the cavity itself.
(A)···Cavities larger than a certain size.
(B)···Cavities smaller than a certain size.
α_1, α_2···, (β_1, β_2···)···Each shows a "BALKEN".
Circles F (F_1, F_2,···), marked with oblique lines···Each shows an SIII-field at its maximum after the administration of fat emulsion.

These facts indicate that if a tuberculous focus is larger than a certain area, some space that every circle (F_1, F_2,···) can not reach, may necessarily remain in the cental part of the focus.

On the contrary, if it is smaller than that certain area (Fig. 19, B), a circle and its neighbours or its opposites may lie on top of each other, so that the central space surrounded by the circles may finally disappear. Furthermore, if the number of "BALKENS" is very small, or if one "BALKEN" is missing in a series, it may result that the circles are separated from each other at that place.

Much toxin from the tubercle bacilli and from the split products of affected tissue has been generally said to be in the central part of a tuberculous lesion which may be outside the reach of SIII-fields. This same circle (SIII-field), however, is definitely the same area which the fat emulsion reaches and where it can neutralize tuberculotoxin and control the growth of tubercle bacilli. Therefore, when there is a space between one circle and another, the two types of toxic action can begin to invade the outside wall of the circles, after filling the space between them. Furthermore the resistance of the fibrous capsules, which the toxic action may attack more or less, may cause it to take a horizontal direction between the row of circles and the capsule.

During this stage, new "BALKENS" may be produced in the next outer layer of the lesion. As they are produced, new circles may appear and the old "BALKENS" may remain as a mould in the necrotic area. The repetition of such a phenomenon may be followed by the appearance of a fat depot in concentric circles as the form of tuberculous lesions in an earlier stage.

When "BALKENS" are not produced in sufficient numbers, the circles, too, can hardly be seen. This probably occurs in early tubercles. The factors mentioned above help to elucidate JOEST's observation and other similar ones as may be seen

below.

No fatty substances are demonstrated in early miliary tubercles, but some time later, they are found in the central part of lesions before necrosis sets in. Furthermore in old lesions with central necrosis, they are found in the border between necrotic and non-necrotic areas. Of course, tuberculous lipemia should affect the foci in a similar manner to the penetration of infused fat emulsion. Finally, it is very probable that after the injection not only of fat emulsion, but also of other preparations, especially anti-tuberculosis drugs, they penetrate partly into and are distributed through some tuberculous lesions at some stage.

VI CONCLUSION

Up to the present, many indistinct points have remained in regard to the basis for the universally-held opinion that fat nutrition is effective to some degree against tuberculosis. The writer has tried to examine these points by observing how experimental pulmonary tuberculous cavities behave in rats receiving long-continued injections of fat emulsion, made by HIKASA.

The results are summarized as follows:

(I) In general, roentgenographic and gross findings: The experimental rabbits almost always have much more general energy than the controls, and also the former's body weight nearly always shows a marked tedency to increase. The tuberculous foci become shrunken and consolidated to a high degree.

(2) Histochemically, at least a part of the infused fat emulsion definitely escapes from the diseased blood vessels of the necrobiotic area in the cavity and later penetrates into and is distributed over the boundary zone of the caseous area.

(3) Histochemically, the characteristics of tubercle bacilli living in a cavity can be changed quite markedly.

(4) Histochemically and roentgenologically, calcification of cavities can be promoted markledy.

(5) Histochemically, the proliferation of argyrophilic fibers occurs more rapidly in the cavity wall at an early stage and they become collagenous early.

(6) Besides the healing tendencies stated above, no direct or indirect side actions of the infused fat emulsion could be recognized. Fatty liver formation and fat embolus formation were especially absent, in spite of the fact that injections had been carried out for each rabbit in groups K(F) and G(F) at least 210 times.

(7) Finally it can be definitely stated that the use of the seame oil emulsion made in our laboratory is more effective against tuberculosis than not using it. In addition, it may be stated that the findings described above show a part of the mechanism of infused fat emulsion, i. e., the action of fat nutrition in tuberculosis.

In closing, the writer wishes to thank Prof. Dr. CHUZO NAGAISHI and members of his staff of the 4th Division of the Tuberculosis Research Institute, Kyoto University, for technical guidance and a gift of the strain of tubercle bacilli (H37 Rv), at the start of this study. The writer is also grateful to Dr. YORINORI HIKASA for his valuable

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脂肪負荷時の実験的家兎結核空洞の態度

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従来から結核症に対して脂肪投与はある程度の効果を有するといわれておりながらもその作用機序は殆ど解明され得たとはいえない現状であるが，私は家兎肺に確実に実験的結核性空洞を作成して教室創製の脂肪乳剤を経静脈性に連続投与し，この点の解明を試みた．

即ち家兎肺の実験的結核性空洞に対する我々の乳剤注射は結果的にみて，

1) レ線並に肉眼所見上，明らかに病巣の萎縮，硬化を促し，体重は上昇傾向を示す．

2) 組織化学的に少なくとも注入脂肪の一部は仮性壊死層の病的血管から確かに漏出して乾酪物質周辺部に滲透分布する．

3) 斯る空洞の領域に於ては結核菌の生活環境を可なり改変させる．

4) 空洞へのカルシウム沈着を確かに促す．

5) 空洞壁の好銀性線維増殖をより早期に惹起せしめ，その膠原化を促す．

6) 上記のような空洞の治癒傾向を示す他に，直接或は間接に何等の副作用も認め得なかつた．脂肪乳剤注入群各家兎につき最少210回の注射に拘らず，特に脂肪肝，脂肪栓塞の発生を立証し得なかつた．

7) 以上の事実から結核症に対して我々の胡麻油乳剤の投与はそれを投与しないよりは好ましいということが確かにいい得る．更に上記の所見は結核症に対する脂肪乳剤注入の作用機序即ち結核症に対する脂肪投与の効果の一端を示すものといいえよう．