

THE INFLUENCE OF FRESH AND AUTOLYZED ORGAN EXTRACTS ON EXPERIMENTAL TUBERCULOSIS.*

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The answer to the question why tubercle bacilli of the bovine type have never been found as the sole agent of tuberculous infection of the human lungs has not been satisfactorily found. It has been assumed that all human pulmonary infection caused by the tubercle bacillus occurs by way of the respiratory tract, and that infection by this path is usually from a pre-existing human source, and consequently the type of organism recovered from these cases is of the human type. There are, however, certain weaknesses in this assumption which justify a doubt as to its validity. It is generally conceded today that tubercle bacilli of the bovine type have the power to infect the human body (Weber and Taute, Oehlecker, British Commission, Th. Smith and Brown, C. W. Duval, Park, W. H. Lewis, A. Hess, Hohlfeld, Moss). There is some question as to the mode of entrance of the bovine type of bacillus to the body, although such entrance is possible, probably by the tonsil (Th. Smith), through the gastro-intestinal tract (Hess, Ravenel, Moss), through injury (Ravenel), or by the respiratory tract (Vagedes). Whatever the portal of entry, they infect mainly children, and once in the human body cause tuberculous lesions chiefly in glands, bones, and meninges. It is quite reasonable to suppose that, having once gained an entrance to the body, bovine bacilli have the same chance of reaching the lung tissues as those tissues where they chiefly lodge and produce disease. In fact, if as Ravenel, Schlossman, and Engel have shown, tubercle bacilli pass readily into the thoracic duct from the intestinal tract, and if the work of Calmette and Guérin, Bongert, Heymann, and Whitla contains any truth, the first capillary system reached by the bacilli is that of the lungs. They would be retained there for a longer or shorter period with the possibility of producing tubercles. Not only the pulmonary capillary system, but also

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those of the spleen and liver would act in a similar manner, as experiments by Ellinger, Besançon and Labbé, Oehlecker, Neumann and Wittgenstein tend to show. There is, however, as indicated, the possibility of lung infection and yet the bovine bacillus has never been recovered as the sole infecting agent from the human lung, the seat of tuberculosis.

The suggestion is frequently made that the tubercle bacillus is changed in type by the long sojourn in the body. Two potent objections make this seem unlikely: First, the strong adherence to type which persists in artificial cultivation (Th. Smith) as well as on the sojourn in the animal body (Weber, Baldwin). Second, that the bovine bacillus has been recovered not infrequently from tuberculosis glands in adults (Lewis, Park, Hess). However, this still leaves certain phases of the question open. Artificial culture cannot answer all the demands of growth in the animal body, and we have many striking examples of necessary caution in drawing conclusions from this source of evidence; for example, the changes occurring during animal passage and artificial cultivation in the other great enemy of lung tissue, the pneumococcus (Rosenow). It is also possible that the tubercle bacilli recovered from glands in adults may be due to a more or less recent infection. Or it may be possible that certain tissues of the body have the power of altering a microorganism while others possess no such power: (See Bail, anthrax bacillus, Tsuda, typhoid bacillus.) Two apparently successful attempts to alter the type of virulence of tubercle bacilli are those of Eber, who, after three years of effort, apparently changed the human into the bovine, and those by Olaf Bang, of changing bovine tubercle bacilli into the avian type. Here, however, the possibility of the animal having contracted tuberculosis of another type after the experimental injection must be borne in mind.

Recent experiments by Calmette and Massol seem to bear on the question of a closer relation than is usually allowed between the bovine and the human type of bacilli. They found that serum from animals immunized against the bacillus of the bovine type had the power of precipitating extracts of bacilli of bovine and human types alike. The criticism may fairly be offered here that

the precipitates may have resulted from certain albuminoids remaining in the extract from the culture medium.

The sensitiveness of tuberculous body cells alike to tuberculin of bovine and human bacillary origin is not admissible here as argument, as such a body may be the seat of infections by both types of bacilli.

The frequency with which children are found to be the hosts of tuberculous infection (51.8 per cent, etc.; Hohlfeld, Shaw, von Pirquet), however, has some bearing on the point, because we know that a fair percentage of these infections are bovine in type (20–25 per cent). This means that, in at least some instances, the infecting organisms which may remain latent for many years may later produce tuberculosis in the body.

Numerous experiments by Rabinowitch, Weichselbaum, Bartel, and others on the virulence of tubercle bacilli in apparently normal or more or less altered organs, have shown how difficult it is to judge the question of virulence of bacilli which have remained latent in the different tissues of the body for various lengths of time.

The question still remains open whether in a child infected previously by the bovine type of bacillus and which later develops the human form of tuberculosis, the new manifestations are to be attributed to a new infection with the human type, or whether the bovine bacilli have in the meantime assumed the characteristic features of the human form.

Recent observations have shown the important rôle of the different tissues of the body in changing the virulence and morphologic characters of the tubercle bacillus. After investigations bearing on the influence of normal blood serum on experimental tuberculosis (White and Graham), we concluded to study the influence of organ extracts on bovine bacilli. It might be possible by such work to account in some measure for the failure to find the bovine bacillus in cases of human pulmonary infection. We approached the question from the side of the possibility of lung tissue having some power of altering the organism used. The experiments here reported deal only with the alteration in the virulence of the bovine organism for rabbits.

TECHNIC.

The extracts were prepared from organs removed from rabbits under aseptic precautions. The organs were washed with sterile salt solution through the vessels to remove the blood as much as possible. They were then cut and reduced in a mortar to a pulp (with or without sterile glass pearls or sand) according to the consistency of the organ used. The material thus obtained was put into a sterile flask and shaken; 0.5 c.c. of this mixture was drawn into a sterile syringe and mixt with a definite weight of emulsion of tubercle bacilli. The balance of the material was placed in the incubator for autolysis for one to three days. In the different series 0.5 c.c. of the autolyzed mixture was taken up in a sterile syringe and mixt with a constant definite weight of bovine bacilli in emulsion. The bovine bacilli used were made with three-weeks'-old subcultures of the strain (B1) obtained through the kindness of Dr. Baldwin of Saranac Lake. The tubercle bacilli for each series were weighed in total quantity for the number of animals used in the series. This quantity was then triturated in a sterile mortar with sterile saline solution, so that each 0.5 c.c. of the emulsion corresponded to 0.003 gm. of moist weight of the original culture. Before each injection the emulsion was triturated and immediately the quantity corresponding to 0.003 gm. of bacilli was drawn into the sterile syringe. To this was added 0.5 c.c. of the fresh or autolyzed organ extract. These suspensions of bacilli and extracts after thorough mixture were then placed in the incubator at 38° C. and allowed to remain for one hour. Each mixture was then injected intraperitoneally into a rabbit.

All extracts were used both before and after autolysis. As seen from series C and D, 1 per cent thymol was added to the organ extracts, both fresh and autolyzed. In the course of our experiments, which extended over 16 months, we obtained in 5 series results that were not uniform. Certain of the results, however, were so striking that a report seems justified.

We were familiar at the start with the interesting work of Bartel and Neumann on the influence of lymphatic extracts on infection of animals with tubercle bacilli. Since our work was commenced the publications of Deycke and Much, Loewenstein, Rabinowitch, Levierato, Weichselbaum, Fontes, Wittgenstein, Trudeau, and Krause have appeared. In our later series we used as a comparison bacilli treated with other extracts (of kidneys, spleen, and nervous tissue) as well as lung and liver extracts. In series E we inclosed the bacillary emulsion in thin capsules of celloidin which were imbedded in the different, washed organs and left for autolysis at 38° C. for 36 hours. After this time the content of the capsule was drawn up in a sterile syringe and injected intraperitoneally.

EXPERIMENTS.

The most striking thing in series A (see Table 1) is the extreme difference between the infection in the autolyzed lung and liver animals and the other animals. The difference between the four other animals was not very marked. It will be noticed that the control lost steadily in weight as did also the fresh liver extract and the fresh lung extract animals. The autolyzed lung animal gained steadily in weight. The autolyzed liver animal lost in all 50 gms.; while the normal serum animal gained 40 gms.

Series B¹ is an exact repetition of series A. The six rabbits used were not all

¹ We wish to acknowledge the work of Dr. D. L. Graham in carrying out the first two series of experiments.

TABLE 1.
SERIES "A."

	A 6 Control 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Salt Solu- tion	A 5 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Rabbit Serum	A 4 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Fresh Liver Extract	A 3 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Fresh Lung Extract	A 2 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Autolyzed Liver Extract	A 1 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Autolyzed Lung Extract
Date of injection	June 21, 1909	June 21, 1909	June 21, 1909	June 21, 1909	June 21, 1909	June 21, 1909
Weight on injection	1,350 gms.	1,310 gms.	1,260 gms.	1,320 gms.	1,260 gms.	1,320 gms.
Life after injection	21 days, died	24 days, killed	24 days, killed	23 days, died	25 days, died	24 days, killed
Weight at death	900 gms.	1,350 gms.	870 gms.	820 gms.	1,210 gms.	1,385 gms.
Number of organs involved	7 2+	5 +	10 +	7 +	4 0	2 0
Tumor formation..	Innumerable	Innumerable	Innumerable	Innumerable	Many single	Few scat- tered tu- bercles in omentum and small intestine
Miliary tubercles..						
Lung involvement	Innumerable	Moderate	Innumerable	Moderate	Moderate	

TABLE 2.
SERIES "B."

	B 12 Control 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Salt Solution	B 11 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Normal Rab- bit Serum	B 10 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Fresh Liver Extract	B 9 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Fresh Lung Extract	B 8 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Autolyzed Liver Ex- tract	B 7 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Autolyzed Lung Ex- tract
Date of injection	June 21, 1909	June 21, 1909	June 21, 1909	June 21, 1909	June 21, 1909	June 21, 1909
Weight on injection	1,370 gms.	1,870 gms.	990 gms.	1,195 gms.	720 gms.	1,095 gms.
Life after injection	19 days, killed	19 days, killed	19 days, killed	19 days, killed	6 days, died	19 days, killed
Weight at death	1,050 gms.	1,920 gms.	620 gms.	820 gms.	480 gms.	1,415 gms.
Number of organs involved with tuberculosis....	9	7	7	10	Died too soon for compari- son	0
Tumor formation..	+	2+	+	+	0
Miliary tubercles..	Innumerable	Innumerable	Less than control	Innumerable	0
Lung involvement	Innumerable	Less than control but con- glomerate tubercles	Less than control	Innumerable	0

of the same litter and were of different weights. In this series the difference between the autolyzed lung animal and all others of the series is more striking, in that the animal in question presented absolutely no sign of tuberculous infection. The autolyzed liver animal died too soon to offer any comparison. The other animals do not

differ materially from each other. The control and the fresh lung and liver extract animals lost steadily in weight. The autolyzed lung extract animal lost 95 gms. and then gained 415 gms. The normal serum animal gained in all 50 gms.

Series C consisted of ten rabbits. Four only are given in Table 3, the remaining six for reasons given below constitute series D. In this series the clumps of bacilli in the emulsion were large and difficult to separate, and the suspension was consequently centrifugalized. This probably resulted in a smaller dose of bacilli for each animal. There was some suspicion of the purity of the culture which had been growing through the summer. Whatever the explanation, the animals of the whole series did not develop the expected degree of sickness.

TABLE 3.
SERIES "C."

	C 1 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Salt Solution	C 2 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Thymol Autolyzed Lung Extract	C 4 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Autolyzed Saline Lung Ex- tract	C 15 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Fresh Liver Extract
Date of injection...	November 16, 1909	November 18, 1909	November 18, 1909	November 16, 1909
Weight on injection...	2,030 gms.	1,460 gms.	1,680 gms.	1,410 gms.
Life after injection...	35 days, killed	179 days, killed	2 days, died	168 days, killed
Weight at death...	2,090 gms.	2,600 gms.	1,700 gms.	2,330 gms.
Number of organs involved.....	3	1	Died too soon for comparison	1
Miliary tubercles ..	Few tubercles on great omentum, diaphragm, and lungs	Fibrinous plaque on the perito- neum. No tu- bercles	0
Lung involvement .	Many tubercles	0	2 tubercles

In this series the lung autolyzed animal died from unknown cause at the end of two days. The lung thymolized autolyzed animal presented less infection than the control animal, as did also the fresh liver extract animal.

The control animal lost 380 gms. and then returned to 60 gms. more than its first weight. The autolyzed thymol lung extract animal had two pregnancies. It gained steadily in weight until one week following its parturition, and then steadily declined.

The fresh liver animal had one pregnancy and gained steadily in weight with the exception of the parturition period.

Series D consisted of six animals of series C and one control. The six animals of this series had received the following injections: Tubercle bacilli treated with extract of lung in salt solution, fresh extract, autolyzed lung extract in salt solution, lung thymol, fresh extract, autolyzed thymol liver extract, fresh thymol liver extract, and autolyzed liver extract in salt solution. These six animals and the one control were injected with 0.003 gm. pure bovine culture 120 days after the injection as indicated in series C, Table 3.

Of the reinjected animals (see Table 4), the autolyzed liver extract animal developed less tuberculosis than the control. It gained in weight in the period following the first injection, then declined steadily and died 6 days earlier than its control. It lost 600 gms. after the reinjection as compared with the 780 gms. lost by the control.

The autolyzed thymol liver extract animal developed less tuberculosis than the control animal, and died 8 days earlier. It gained steadily in weight until the reinjection and then lost 620 gms. The thymol fresh liver extract animal developed as much tuberculosis as the control; was killed one day later; it had one pregnancy and lost after the reinjection 270 gms.

TABLE 4.
SERIES "D."

	Control 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Salt Solu- tion	D 5 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Fresh Lung Ex- tract	D 13 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Autolyzed Lung Ex- tract	D 16 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Thymol Fresh Lung Ex- tract	D 18 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Autolyzed Thymol Liver Ex- tract	D 17 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Thymol Fresh Liver Ex- tract	D 20 0.003 gm. Bovine Tubercle Bacilli +0.5 c.c. Autolyzed Liver Ex- tract
Date of first injection.....	March 17, 1910	Nov. 16, 1909	Nov. 18, 1909	Nov. 16, 1909	Nov. 18, 1909	Nov. 16, 1909	Nov. 18, 1909
Weight on injection	1,950 gms.	2,000 gms.	1,900 gms.	1,450 gms.	1,130 gms.	1,030 gms.	1,230 gms.
Date of second injection.....	March 17, 1910	March 17, 1910	March 17, 1910	March 17, 1910	March 17, 1910	March 17, 1910
Weight on second injection.....	2,700 gms.	2,170 gms.	2,500 gms.	1,970 gms.	1,080 gms.	1,060 gms.
Life after injection..	35 days, died	162 days, killed	148 days, died	146 days, died	147 days, died	162 days, killed	146 days, died
Weight at death...	1,170 gms.	2,370 gms.	1,830 gms.	1,860 gms.	1,350 gms.	1,710 gms.	1,300 gms.
Number of organs involved.....	18	14	14	15	13	18	14
Tumor formation...	1	2	2	2	4	3	3
Miliary tubercles...	Innum- erable	Less than control	Innum- erable on dia- phragm, lungs, and large omen- tum	Innum- erable in most organs. A little less in liver, dia- phragm, pericar- dium, and sex organs	Innum- erable, ex- cept in perito- neum, kidneys, and me- sentry	Innum- erable. No marked differ- ence from control animal	Innum- erable, ex- cept in large in- teste- ne and thoracic glands where less
Lung involvement..	Innum- erable	Less than control	Innum- erable	Innum- erable	Innum- erable	Innum- erable	Innum- erable

The thymol fresh lung extract animal developed less tuberculosis than the control; died 9 days earlier; had one abortion after reinjection, and lost 640 gms. The saline autolyzed lung extract animal developed less tuberculosis than the control; died 2 weeks earlier and lost 340 gms. After the reinjection, the saline fresh lung extract animal developed less tuberculosis than the control; lived 7 days longer, and lost 330 gms.

If any conclusion be justified from this series, it would seem that the previous injection rendered the animals less resistant to the reinfection, for while all developed less tuberculosis and lost less in weight, yet they succumbed more readily to the infection.

Series E consisted of 5 animals; the suspension of 0.003 gm. of tubercle bacilli was inclosed in a thin celloidin capsule and imbedded in the freshly removed and washed organs (lung, liver, kidney, and spleen) which were allowed to undergo

autolysis, in a moist chamber, in the incubator at 38° C. for 36 hours. After autolysis, the suspension of tubercle bacilli was withdrawn from each capsule in a sterile syringe and injected intraperitoneally in a rabbit. The control received 0.003 gm. of tubercle bacilli which had been enclosed in a celloidin capsule in a moist chamber in the incubator for the same length of time. This was then withdrawn with a sterile syringe and injected intraperitoneally. In this series the amount extracted from the spleen and kidney capsule was less than that from the control and the lung and liver capsules.

We feel that owing to the fact that the amount of bacillary emulsion withdrawn from the capsule was not the same for each animal, no definite comparison of the weight of bacilli introduced could be made. This experiment was unsatisfactory; nevertheless, it is striking that none of the animals which received tubercle bacilli after being subjected to the action of autolyzing organs developed as much tuberculosis as the control, and especially that the lung capsule rabbit was the only one which gained in weight.

TABLE 5.
SERIES "E."

	Control Content of Control Capsule Corresponding to 0.003 gm. Bovine Tubercle Bacilli	E Lg. Content of Lung Capsule 0.2 c.c. Diluted with 0.4 c.c. Sterile Salt Solution	E Lv. Content of Capsule Kept in Autolyzing Liver + 0.15 c.c. Bovine Bacilli + 0.4 c.c. Salt Solution	E K. Content of Capsule from Autolyzing Kidney + 0.015 c.c. Bovine Bacilli + 0.2 c.c. Sterile Salt Solution	E Sp. Content of Capsule from Autolyzing Spleen + 0.2 c.c. Sterile Saline Solution
Date of injection . . .	February 5, 1910	February 5, 1910	February 5, 1910	February 5, 1910	February 5, 1910
Weight on injection . .	2,550 gms.	2,360 gms.	2,330 gms.	2,240 gms.	2,330 gms.
Life after injection . .	40 days, killed	40 days, killed	40 days, killed	40 days, killed	104 days, killed
Weight at death . . .	2,330 gms.	2,530 gms.	2,200 gms.	2,140 gms.	2,260 gms.
Number of organs involved	15	8	6	2	8
Tumor formation . . .	2	0	0	0	0
Miliary tubercles . . .	Innumerable	Much less than control	Few tubercles in omentum, small intestine, diaphragm, and lungs	Few tubercles only in kidneys and spleen	Moderate amount
Lung involvement . . .	Innumerable	Very few tubercles	Very few tubercles	None	Few tubercles

In series E we used 8 animals and one control. This series was similar to series A and B with the exception that the fresh and autolyzed extracts of spleen and brain were added. The organs which were used for the extracts in this series were taken from an animal two years old. The animals which were injected with the mixture of bacilli and organ extract were young, varying between two and four months. The extracts were allowed to autolyze for 24 hours only. These three points offer the only explanation we can give for the difference between this experiment and the previous ones, and we would suggest that extracts from an animal of old age, autolyzed for a shorter time, injected into very young animals, coupled with the difference of susceptibility to tuberculous infection at different ages, may offer the reason for the different result.

Both brain extract animals, which were not much different from each other however, were approximately the same as the control animal. For some reason this

TABLE 6.
Series "F."

	Control. 0.003 gm. Bovine Tu- bercle Bacilli +0.5 c.c. Salt Solution	0.003 gm. Bovine Tu- bercle Bacilli +0.5 c.c. Fresh Lung Extract	0.003 gm. Bovine Tu- bercle Bacilli +0.5 c.c. Au- tolyzed Lung Extract	0.003 gm. Bovine Tu- bercle Bacilli +0.5 c.c. Fresh Liver Extract	0.003 gm. Bovine Tu- bercle Bacilli +0.5 c.c. Autolyzed Liver Extract	0.003 gm. Bovine Tu- bercle Bacilli +0.5 c.c. Fresh Spleen Extract	0.003 gm. Bovine Tu- bercle Bacilli +0.5 c.c. Autolyzed Spleen Extract	0.003 gm. Bovine Tu- bercle Bacilli +0.5 c.c. Fresh Brain Extract	0.003 gm. Bovine Tu- bercle Bacilli +0.5 c.c. Autolyzed Brain Extract
Date of injection.....	April 4, 1910	April 4, 1910	April 5, 1910	April 4, 1910	April 5, 1910	April 4, 1910	April 5, 1910	April 4, 1910	April 5, 1910
Weight on injection.....	2,880 gms.	1,810 gms.	2,030 gms.	1,100 gms.	1,030 gms.	1,300 gms.	1,020 gms.	1,000 gms.	970 gms.
Life after injection.....	39 days, killed	40 days, killed	37 days, died	31 days, died	44 days, killed	43 days, killed	43 days, killed	45 days, killed	44 days, killed
Weight at death.....	2,080 gms.	1,545 gms.	1,130 gms.	920 gms.	1,810 gms.	1,720 gms.	1,060 gms.	1,800 gms.	1,530 gms.
Number of organs involved.	14	16	16	14	5	1	16	11	14
Tumor formation.....	2	2	2	3	1	0	1	1	2
Miliary tubercles.....	Innumerable	Much less than con- trol	Innumerable	Innumerable	Very few	0	Innumerable, even more than con- trol	Innumerable, a little less than con- trol	Innumerable, not much different from con- trol
Lung involvement.....	Innumerable	Innumerable	Innumerable	Innumerable	Few conglomerate	0	Innumerable	Innumerable, not so many as in control	Less than con- trol

does not bear out fully the experiments of Deycke and Much. The autolyzed lung and fresh liver animals died spontaneously two and eight days earlier than the control. The autolyzed liver extract, the fresh spleen extract, the autolyzed spleen extract and both brain extract animals gained steadily in weight. The control animal, both lung extract and fresh liver extract animals steadily declined. The animals that gained in weight, with the exception of the fresh liver animal, were about one month and a half younger than the other three animals.

GENERAL SUMMARY.

As a result of our experiments it would seem that under certain conditions extracts of autolyzed rabbits' lungs contain a factor which when incubated with bovine tubercle bacilli changes their virulence (series A, B, C, D).

The explanation of this phenomenon is difficult with our present knowledge. It is apparently not due to a dissolution of the bacilli, as smears made from the mixture of bacilli and extract after incubation show the bacilli very little changed in form and staining power. It may be due to the reaction of the extract which is alkaline from the beginning to as late as three weeks following the beginning of autolysis. As pointed out by Koch and later by Rabinowitch, an alkaline medium has a marked influence on the staining properties and virulence of tubercle bacilli. We do not feel, however, that the alkalinity of the extract is the sole factor to be considered in explanation of these results because the extracts of other organs during autolysis became alkaline (spleen and brain), and yet these did not produce the same reduction in virulence of the tubercle bacilli although the condition of the preparation was the same for all extracts made by us.

Another possible explanation of the influence of the autolyzed lung extract may be found in the presence of lipoid bodies in the lung (lecithin, neurin, and cholin). This would bring the experiments into the same category as the experiments of Deycke and Much, who believe that the influence of brain substance on tubercle bacilli in their experiments was due to the presence of neurin and cholin in the extracts. This latter statement, however, is doubted by Loewenstein, F. Jessen, and Rabinowitch, who ascribe the changes found by Deycke and Much to error in the technic and to the alkalinity of the solution as pointed out by

Koch in his original monograph. Lecithin, however, is probably present during the lung autolysis and we were able to demonstrate cholin crystals in our lung extracts (autolyzed for 3 days) by the method of Rosenheim.

Another possible explanation might be found in the action of nucleinic acids directly on the tubercle bacilli or indirectly by stimulating the lymphocytes of the body to a more vigorous defense (Neumann, citing von Bartel, Bacherach, and Stein).

Another explanation, which, however, does not seem to us very likely, may be found in the action of fatty acids and of soaps. The experiments of Noguchi have shown the influence in certain concentrations of oleic soaps on the virulence and growth of tubercle bacilli. The fats contained in lung tissue may undergo autolysis and be split into fatty acids and glycerin, and combined with the alkaline radicals may act on the tubercle bacilli. The alkaline reaction throughout the autolysis of lung tissue, however, would not support this view.

Another possible explanation might be found in the action of specific enzymes contained in autolyzed lung tissue and the action of these on the tubercle bacilli. Although Opie and Barker and Berger have demonstrated a lipase in lymphocytes, and Magnus Levi in the liver, the presence of such a lipolytic ferment in lung tissue has not been observed. Simon in working with autolyzed lung tissue found a fair amount of neutral fat in large and small drops. Bartel obtained his favorable results also with boiled organ extracts. A series of heating experiments now in progress in this laboratory render the enzyme explanation unlikely as does also the fact that we could find little change in smears made after the action of the extract of autolyzed lung on the bacilli.

It may be possible that the change in virulence is due to the presence of other symbiotic bacteria which grow in the tissue during autolysis. The experiments of Rosenbach, who observed that tubercle bacilli may lose in virulence if grown together with trichophyton, lend some support to this view. Further, Vaudremer has lately published observations on the influence of *Penicillium glaucum* and *pyocyaneus* in diminishing the toxic power

of tuberculin. Applied to our experiments, however, it seems unlikely since the other organisms were present in the extracts of all autolyzed organs alike.

Provided that these experiments are applicable to all animals it is interesting that the lung tissue, which is the seat of maximum attack of the bacillus during the life of the animal, should develop during autolysis a substance which has so marked an influence in reducing the virulence of the germ for other animals of the same species. Cadeac and Mallet found the tubercle bacilli still virulent after 102 days in lung tissue that had been desiccated and powdered and after 17 days in lungs that had been powdered and decomposed. Schottelius found virulent tubercle bacilli in lungs that had been buried for $2\frac{1}{2}$ years. Petri found virulent bacilli after 22 and 96 days in rabbits that had been buried in wooden and tin coffins. Loesener obtained tubercle bacilli which would produce general tuberculosis from lungs that had been buried for 60 days, and local tuberculosis from lungs that had been buried for 95 days. Bartel and Hartel, however, found that bovine tubercle bacilli after desiccation in rabbits' lungs for 9 days lost their virulence.

The length of time allowed for autolysis would seem to be a factor of great importance since the phenomenon was not obtained in lung tissue which was allowed to autolyze for 24 hours only, but was most marked after autolysis had progressed for three days. The autolysis of lung tissue follows a different and a slower course than the autolysis of other organs, as shown by Jacoby and Johannsen. The latter found that all organs, except the lungs, are able to reduce an aqueous solution of methylene blue.

The results obtained with the extracts of autolyzed liver are in accord with the investigations of Bartel and Stein and Neumann and Wittgenstein, who were able to reduce by these extracts the virulence of tubercle bacilli. Our results with liver extract, however, are not to be compared with the results in our lung animals. In this connection it is to be noted that the liver extract is acid from the beginning (Jacoby, Magnus Levi, Salkowsky, Bartel, Schryver), due to the presence of acids (lactic acid and, from the odor of our extracts, probably of butyric and acetic acid), thus

differing markedly from the lung extract which was alkaline throughout.

The two experiments with the extracts of the spleen and brain are not enough to justify any conclusions. The brain animals, while they increased steadily in weight, did not confirm the favorable results of Deycke and Much, as there were almost as many conglomerated and single tubercles found as in the control animal. The acid brain medium suggested by Ficker for the cultivation of tubercle bacilli would not support the view of diminished virulence of tubercle bacilli by the action of brain extract. The reaction of the brain extract at the beginning of autolysis was almost neutral and during the three days of autolysis became alkaline, though the tubercle bacilli subjected to the influence of this extract did not show great changes in their character or virulence.

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