Definitely positive tests appear to be associated with evidence of clinical improvement, despite the presence of a tuberculous condition which might be classified as far advanced.

Skin reactions with Berkfeld filtrates are probably a measure of the resisting capacity of the patient or of normal persons, and as such are indicative of antibody content in the tissues.

The importance of evaluating the clinical course of groups of tuberculous patients must be emphasized in any attempt to interpret the significance of such skin reactions.

Further studies are now in progress with Berkfeld filtrates of tubercle bacillus cultures to determine toxicity and immunizing properties. More extensive series of tuberculous patients and normal persons are being studied along the lines indicated in this progress report.

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Thermolabile Toxin and Thermostable Tuberculin Components in Berkfeld Filtrates of Broth Cultures of Tubercle Bacilli.

FREDERICK EBERSON.

From the Department of Medicine and George Williams Hooper Foundation for Medical Research, University of California.

In these experiments, normal and tuberculous guinea pigs were used to determine by intracutaneous tests whether or not Berkfeld filtrates prepared from broth cultures of tubercle bacilli contain toxins which might be distinguishable from tuberculin elements. One series of tuberculous animals had been infected 3 weeks prior to the test and showed early tuberculosis with little, if any, toxemia. The other group of animals, infected 4½ months previously, showed well marked signs of advanced tuberculosis.

A human strain of virulent tubercle bacilli was inoculated into ordinary infusion broth containing 5 per cent glycerine. The Berkfeld filtrates were prepared from a 26-day old culture grown at a temperature of 38° to 40° C. One lot of the filtrate was heated at a temperature of 63° to 65° C. on a water bath. Both portions were preserved with 0.25 ether tricresol. At the time the experiments were made, the material had been stored in the ice-chest for 4 days.

The animals were injected intracutaneously with 0.03 cc. of the heated and unheated filtrates, respectively, in a dilution of 1:2 (with

TABLE I. *Intracutaneous Reactions with Berkfeld Filtrates of Human Tubercle Bacillus.*

Guinea Pigs	Heated Filtrate* (Diluted 1:2)		Unheated Filtrate (Diluted 1:2)		Filtrate and Normal Guinea Pig Serum†		Filtrate and Serum from Tuberculosis Guinea Pig†	
Normal	24 Hrs.	0.2x0.2 cm.; slight infilt. and reddening	24 Hrs.	0.75x0.75 cm.; marked infilt. and reddening	24 Hrs.	Negative	24 Hrs.	0.25x0.25 cm.; slight reddening and slight infilt.
	48 Hrs.	Reaction lessened	48 Hrs.	0.4x0.4 cm.	48 Hrs.	Slight reddening	48 Hrs.	0.2x0.2 cm.; slight reddening
	72 Hrs.	Faint reaction		3rd to 6th day still positive		Negative on 3rd day	72 Hrs.	Slight reddening
		Negative with residual pigmentation, on 4th day		Negative on 7th day				Negative on 5th day
Early Tbc. (21 day inf.)	24 Hrs.	0.5x0.5 cm.; slight reddening and slight infilt.	24 Hrs.	1.0x1.0 cm.; deep infilt., reddening and cent. zone 0.5x0.5 cm.	24 Hrs.	0.25x0.25 cm.; slight reddening	24 Hrs.	0.5x0.75 cm.; reddening and slight infilt. with erythematous border
	48 Hrs.	Reaction lessened					48 Hrs.	0.25x0.25 cm.; reddening and scarring
	72-144 Hrs.	Still positive				Negative on 2nd day		Negative on 4th day
		Negative on 7th day		Strongly positive on 7th day				
Advanced Tbc. (4½ mo. inf. with toxemia)	24 Hrs.	1.0x1.0 cm.; blanching and slight infilt.	24 Hrs.	1.5x1.0 cm.; blanching and erythematous border; central purplish zone; reddening and infilt.	24 Hrs.	1.5x1.0 cm.; edema, reddening, infilt. and cent. purplish zone	24 Hrs.	1.0x1.0 cm.; blanching and edema; cent. purplish zone with marked erythematous border of 2-3 cm.
	48 Hrs.	1.2x1.2 cm.; blanching and slight infilt.	48 Hrs.	1.25x1.25 cm.; deep infilt. and same picture as 24 hrs.	48 Hrs.	1.5x1.5 cm.; as above with deep infilt. and attachment to underlying tissue	48 Hrs.	1.0x1.0 cm.; same as preceding day
	72 Hrs.	1.0x1.0 cm.; slight reddening and slight infilt. with cent. grayish zone	72 Hrs.	1.0x1.0 cm.; same as preceding day	72 Hrs.	1.0x1.0 cm.; very marked reddening, infilt. and cent. grayish zone 0.3x0.3 cm.	72 Hrs.	0.5x0.5 cm.; infilt. and small cent. zone
	7th Day	Slightly positive reaction	7th Day	Reaction is still strongly positive	7th Day	0.5x0.5 cm.; less reddening and infilt.	5th Day	0.5x0.5 cm.; reddening and deep infilt.
		Negative, on 14th day with residual pigmentation		Negative, on 14th day, with residual pigmentation	10th Day	Reaction is still positive	7th Day	0.3x0.4 cm.; reduced infilt. and reddening
						Negative on 17th day	10th Day	Reaction is faintly positive
								Negative on 17th day

*Material was heated on a water bath at temperature of 63-65° C. for 1 hour.
†Berkfeld filtrate was combined with equal volume of serum and incubated 1 hour at 37° C. on water bath, before injection.

physiological salt solution). Another series of experiments was set up with unheated filtrates combined separately with equal volumes of normal guinea pig serum and with serum from tuberculous guinea pigs, and incubated for one hour at 37° C. on a water bath before injection. The object of these experiments was to determine the effect, if any, which normal and tuberculous guinea pig serum might have upon the reacting properties of the filtrates. These combined materials were injected in 0.03 cc. amounts as in the preceding series and controlled with guinea pig serum alone.

The results of the skin tests showed conclusively that unheated Berkfeld filtrates gave more pronounced reactions and those of longer duration than did the heated filtrates, which were often negative. From the chart it can be seen that the guinea pigs with advanced tuberculosis gave in all cases very positive reactions which persisted for as long as from 14 to 16 days. Again, the group of animals with early tuberculosis gave more pronounced reactions, which were of longer duration, than those observed in the normal animals. Experiments with the filtrates which had been combined with normal and tuberculous guinea pig serum indicated that normal serum exerted some neutralizing action on the toxin element, while the serum from tuberculous guinea pigs did not. Finally, the more decided reactions observed in all the groups of animals injected with filtrates which had been previously combined with tuberculous serum, showed that a tuberculin element had been superadded by the tuberculous serum, while excluding the presence of any neutralizing antibody.

These observations indicate that Berkfeld filtrates prepared from broth cultures of tubercle bacilli contain a heat sensitive toxin-like substance which can be distinguished from the tuberculin element present in ordinary tuberculins. Both the toxin and tuberculin components can be demonstrated at the same time in cultures which have grown for from 3 to 4 weeks. By a simple procedure of heating the filtrate at a temperature of from 63° to 65° C. for one hour on a water bath, the toxin is inhibited or destroyed, while the heat-resistant tuberculin is left free to give typical skin reactions. These observations are in agreement with those reported for sera from tuberculous patients and animals.[1]

It remains to be determined at what point during the incubation period of tubercle cultures the tuberculin element first makes its appearance. This substance can be considered doubtless as a disintegration product of the organisms. In order to be certain that subsequent experiments with Berkfeld filtrates from the standpoint of

pure toxin production be decisive, it will be necessary to establish this point. Obviously there must be a time when toxin production is at a maximum and when the progressive developmental cycle of thermolabile and thermostable components can be studied. To this end experiments are now being made with different strains of tubercle bacilli grown in different media for varying periods of time. Work now in progress with a rapidly growing strain of tubercle bacilli points to the presence of a tuberculin-free thermolabile component which gives skin reactions in normal and tuberculous patients and animals. These results can be correlated not only with the stage of infection but with the clinical progress of the disease. There is clear-cut evidence also that most of the patients with far advanced tuberculosis do not give minimal or negative tests unless antibodies are absent, contrary to the report of Herrold and Saelhof.[2]

[1] Eberson, F., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 79.

[2] Herrold, R. D., and Saelhof, C. C., *J. Am. Med. Assn.*, 1926, lxxxvi, 747.

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A Note on Tissue Respiration in Relation to Thyroidectomy.

G. L. FOSTER.

From the Division of Biochemistry and Pharmacology, University of California Medical School.

In the course of a study of the influence of lactic acid upon tissue respiration it was observed that tissues from thyroidless rats showed a lower rate of oxygen consumption than did similar tissues from normal rats.

Bits of diaphragm tissue suspended in Ringer solution containing 0.2 per cent glucose and buffered to pH 7.5 by a M/80 phosphate mixture under an atmosphere of oxygen were studied by Warburg's[1] manometric method.

Normal.		Thyroidless	
Wt. of animal.	Qo_2* 1st hour.	Wt. of animal.	Qo_2* 1st hour.
gm.		gm.	
142	8.2	174	4.0
145	8.8	175	5.3
172	6.4	241	5.3
175	9.6	177	6.8
180	5.7	155	4.5
mean	7.7	mean	5.2

* Qo_2 = cubic millimeters O_2 per mg. dry tissue per hour.