certain dietary factors (referable to the nature of protein) exert an influence upon the content of muscle creatine. This observation is being further investigated.

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On re-injection with B. tuberculosis or its products and with sera.

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We have stated in several papers read before this Society that extreme vaso-depression was caused by the intravenous injection of split products of bacterial and other origin. These statements were part of an endeavor to elucidate experimentally the mechanism by which foreign organisms and substances may possibly cause intoxication, infection and anaphylaxis by neutralizing pressor secretions, by removing or using up the nourishment of the host, or by destroying the processes upon which depend the host's specificity, vaso-energy and power of reforming foreign bodies into substances like its own constituents, which may be essential in these conditions to its existence.

This view includes the action of those foreign substances and ferments upon the host, by which the host organism or substrata gives off split products which thus produce an auto-intoxication.

These notes carry this view still further by means of a few observations on death and symptoms following re-injection in animals which have already been injected with tubercle bacilli or products of the tubercle bacilli and with sera. An explanation is advanced that the death which followed the re-injection of the minute dose of *B. tuberculosis*, with the long interval between the first and second injection, namely twelve months, is probably due to a deferred anaphylaxis or persistent increased susceptibility or sensitiveness which may be present for an unusually long time in some of the cases which have apparently recovered from the first injection. There were not enough tuberculous lesions found in this case to satisfactorily account for death.

The initial dose according to this interpretation produces a state of responsiveness or sensitiveness which reacts to the attack or action of the re-injection, by the production of free-bonded substances and split products, poisonous secretions or eliminations which result in the intoxication or functional disturbance which may end in death.

A practical application of our observations is made to the administration of therapeutic sera. The re-injection of split products of the *B. tuberculosis* obtained by chemical agents and electrolysis have given interesting results.

PROTOCOL 1: A POSSIBLE DELAYED ANAPHYLAXIS FOLLOWING RE-INJECTION WITH *B. tuberculosis.*

Large black mongrel dog, weight 25 lbs., was injected April 25, 1912, intravenously with 4 mg. No. 634 bovine culture of the *B. tuberculosis* contained in 4 c.c. of physiological saline solution. The dog was sick for about two months, with loss of weight. It then recovered and gained weight and strength and appeared to be in good health. Nine months after this recovery and 12 after the first injection, the dog was apparently normal and had increased in strength and weighed 33¼ lbs. This dog was re-injected on April 16, 1913, with 4 mg. intravenously of a virulent bovine culture (Ravenel) of the *B. tuberculosis*, contained in 4 c.c. of physiological saline solution. This dog appeared well immediately after the re-injection and remained so for ten days. Twelve days after the re-injection bloody mucus stools were observed. It then became quiet and three days later, 17 days after the re-injection, it was found dead with some signs of hemorrhage from the nostrils. The autopsy showed a very few tubercles in the pleura covering the upper lobes of the lungs. The liver was fatty and the lungs extremely congested. The spleen and other organs were negative. The cause of death in this case would possibly appear to be a persistent or delayed constitutional reaction, rather than due to the 4 mg. of the tubercle bacilli per se. The initial dose set up a condition in the host which responded to the re-injection by setting free ferments and split products or secretions, which thus produced an auto-intoxication as outlined in the beginning of this paper.

Control: Small white dog, 12 lbs., received April 25, 1912, an initial injection of 4 mg. of bovine culture No. 634, intravenously in 4 c.c. of physiological saline solution. The dog was sick and lost weight for several months, and then regained its weight. Two superficial abscesses with necrosis of the skin and subcutaneous tissue developed at the point of inoculation, which healed after a few months. This dog was re-injected intravenously April 16, 1913, with 4 mg. of a virulent bovine culture (Ravenel) of the *B. tuberculosis* contained in 4 c.c. of physiological saline solution. The healed ulcers of the initial injection re-opened 12 days after the re-injection as two clean, punched out ulcers and an area of infiltration arose at the site of the re-inoculation. This dog became much emaciated and had a purulent conjunctivitis. No hemorrhage was observed from nostrils or in stools. Respiration 48 per minute, 19 days after the re-injection. Died May 12, 1913. Autopsy showed a typical general miliary tuberculosis, with mediastinal glands enlarged and caseous. The cause of death in this dog was undoubtedly general tuberculosis.

PROTOCOL 2: CONTROLS ON CULTURE NO. 634 (BOVINE).

Mongrel dog, 25 lbs., injected intravenously April 25, 1912, with 4 mg. No. 634 bovine culture of *B. tuberculosis*, was killed June 26, 1912. The autopsy showed a case of typical infection. The lungs contained numerous small tubercles, with no caseation or consolidation. Kidneys, about 300 large tubercles. Liver, a few tubercles. Several other dogs were used to control the bovine cultures, Ravenel and No. 634.

PROTOCOL 3: DELAYED ANAPHYLAXIS.

A small quantity of the insoluble non-toxic portion of the tubercle bacilli, prepared according to Vaughan's method, was injected into each of 2 guinea pigs. After 3 weeks, these two pigs were re-injected, intraperitoneally, with an emulsion of tubercle bacilli, from which the split products had been prepared.

Pig No. 1 showed some restlessness, at the time of re-injection, but gave no other sign that it had been affected by the re-injection. Two days later this pig was seized with a convulsion and died.

Control Pig No. 2: Re-injected as above. 30 minutes after

the re-injection, restlessness followed by weakness in the hind legs; violent convulsions followed by extreme fatigue. The convulsions continued and death followed in a convulsion six hours after the injection.[1]

PROTOCOL 4: RE-INJECTION WITH TUBERCULOUS SERUM FOLLOWING A TUBERCULIN INJECTION.

The subcutaneous injection of 10 c.c. of the serum of a tuberculous rabbit into each of three rabbits, each of which had been previously sensitized by an injection of 1 c.c. of crude tuberculin, caused death in 24 to 50 hrs.[2]

The autopsy showed, as the principal lesion, large, marked areas of intestinal inflammation and necrosis.

If this state is dependent on substances with free bonds or not homogenized, or on split products, as we have outlined, one should be able to detect it by the presence of reactions, changes of blood pressure, precipitins, ferments or similar agents, loose in the fluids or stored in the organs.

It has been shown by one of us that tubercle bacilli suspended in a saline solution are considerably changed upon being electrolyzed in their reaction to stains. If a porous cell such as a Berkfeld filter is used as a septum in a U-tube and suspensions of tubercle bacilli are put in both arms, one portion will be acted upon by chlorine and the other portion by sodium or sodium hydrate as a result of electrolysis. The organisms acted upon by chlorine lose their acid-fast quality when decolorized by nitric acid and substances that have the acid-fast principle may be shaken out of solution with ether. The portion acted upon by sodium or sodium hydrate does not noticeably lose its acid-fast principle. There are other changes which can be demonstrated and which are now being investigated.

A guinea pig was re-injected twice with tubercle bacilli which had been submitted to the above chlorine treatment. The injections were made during a period of two weeks. Within an hour after the third injection the guinea pig showed signs of illness and became steadily worse during the day. It died during the night

[1] A. and F., PROC. OF SOC. FOR EXP. BIOL. AND MED., 1910, VIII, pp. 24–28.
[2] A. and F., PROC. OF SOC. FOR EXP. BIOL. AND MED., 1910, VII, pp. 77–79.

following the third injection. Autopsy showed inflammation and extensive necrosis at the point of injection.

There were no characteristic signs of anaphylaxis observed during the illness of this guinea pig, although this was probably a case of death after sensitization. The electrolytic products of the tubercle bacillus are being further tested as possible immunization agents.

The variable degree of the persistence of sensitiveness in and following infections has led us in the use of sera to inject a very minute initial dose of serum and to repeat with a slightly increased dose instead of using one large dose of the entire quantity to be administered. This method was elaborated by us as shown before this society.[1]

Some observations on the action of ergot.

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The activity of ergot was tested by the cock's comb reaction on white Leghorn roosters, the fluid extract being employed in all the experiments. Alcohol was found to increase the susceptibility to the drug as shown by the decreased activity of the preparation after the alcohol was driven off. Smaller doses of ergot were also required to produce a reaction in acute alcohol intoxication. A fall of temperature was also noticed when ergot was given in this condition, thus indicating a reversible action, since in the normal subject the injection of ergot frequently caused a marked rise of temperature. The repeated injection of ergot at intervals of 24 to 48 hours failed to produce any cumulative effect in the normal subject. But in starvation there was a decided bluing of the comb after the third injection of a sub-minimum dose, the effect becoming more marked with successive injections. In a large number of experiments carried out on normal subjects it was found that when the injections are made at proper intervals the cock's comb reaction

[1] PROC. OF SOC. FOR EXP. BIOL. AND MED., Feb. 21, 1912, IX, pp. 49–51.