

The total extract is injected in 6 doses over a period of 3 days. The rats are killed 100 hours after the first injection. Their ovaries are cut in serial section.

Results. 1. A P R I is noted throughout the cycle to a varying degree. 2. The reaction appears strongest from 6 to 9 days from the onset of the preceding menstruation. 3. From the 6th to 9th day an indication of lutein change is noted. It is too faint to be interpreted as a full A P R III. 4. These results signify that approximately 25 M. U. L. is the maximum amount of Prolan A occurring in the circulation of the non-pregnant woman.

5643

Calcification of Tubercles by Administration of Calcium Chloride.

VALY MENKIN.

From the Department of Pathology, Harvard Medical School.

In previous communications^{1, 2} it was shown that a vital dye, trypan blue, or a metal in the form of its salt, ferric chloride, when injected into the circulating blood stream rapidly accumulates in an area of inflammation, where the substance is fixed and fails to drain to the tributary lymph nodes. Subsequently it was shown in infected rabbits^{3, 4} that repeated daily intravenous injections of ferric chloride are followed by an accumulation of iron in the caseous center of tuberculous areas.

The object of this study was to determine whether calcium would likewise penetrate from the blood stream into tuberculous foci. Such evidence might offer a reasonable explanation for the origin of this element in calcified tubercles. The literature fails to reveal any agreement on the effect of calcium in experimental tuberculosis. Michelazzi⁵ found calcification of lesions in tuberculous rabbits treated with calcium. Hoyle,⁶ who maintained that these earlier experiments were unsatisfactory, found no appreciable difference in the

¹ Menkin, V., *J. Exp. Med.*, 1929, **50**, 171.

² Menkin, V., *J. Exp. Med.*, 1930, **51**, 879.

³ Menkin, V., *Proc. Soc. Exp. Biol. and Med.*, 1930, **27**, 1020.

⁴ Menkin, V., and Menkin, M. F., *J. Exp. Med.*, 1931, **53**, 919.

⁵ Michelazzi, *Gaz. d. Ospedali*, Milano, 1904, **25**, 425; *abst. Rev. de la Tuberc.*, 1904, **1**, 421.

⁶ Hoyle, J. C., *Quart. J. Med.*, 1929, **22**, 451.

course of the disease between experimental and control groups. He, however, failed to make histological comparisons. Maver and Wells⁷ found in guinea pigs that the amount of calcium in tuberculous lesions is not appreciably modified by adding calcium lactate to the usual diet. Spies⁸ recently demonstrated that repeated administration of irradiated ergosterol causes calcification of tubercles.

In our experiments bovine tuberculosis was induced in rabbits by the intravenous injection of 0.01 mg. of a saline suspension of Ravenel strain. From 3 to 4 weeks later several of the rabbits were given daily intravenous injections of 5% CaCl₂ solution. On the first day each animal received 3 cc. of this solution; 4 to 6 cc. was injected on each of the subsequent days. After a variable number of injections these rabbits were killed. The lungs revealed extensive caseous tuberculosis. Some of the caseating areas appeared firmer to touch than usual. The tissues were fixed in 10% formaldehyde. Histological preparations were stained with hematoxylin and eosin. In addition special stains for calcium were made by the silver method of Von Kossa and by 1% aqueous sodium alizarin sulphonate. The latter test was performed in view of studies of Cameron,⁹ who maintains that alizarin is the most reliable test for freshly deposited calcium in tissues. In all the microscopic sections there was complete agreement in the results as obtained by the 3 methods of staining for calcium. To serve as controls several tuberculous animals that received no CaCl₂ were killed at about the same time as the experimental rabbits. The lungs which revealed about the same extent of caseating tuberculosis as in the injected rabbits were studied histologically for the presence of calcium deposits. When calcium was found it was always within the caseous areas either as compact masses or frequently as scattered coarse granular deposits. Inasmuch as spontaneous calcification of tubercles is frequently seen in non-injected rabbits with tuberculosis of long duration, all studies were carried on in animals that had the disease for not longer than 4 to 6 weeks. The number of calcified caseous tubercles per microscopic section were counted. The results are shown in Table I. It is clear that more (10 times as an average) caseous areas with calcium deposits were found in animals repeatedly injected with CaCl₂ than in non-injected animals. Furthermore the degree of calcification was generally more marked in the injected

⁷ Maver, M. E., and Wells, H. G., *Am. Rev. Tuberc.*, 1923, 7, 1.

⁸ Spies, T. D., *Am. J. Path.*, 1930, 6, 337.

⁹ Cameron, G. R., *J. Path. and Bact.*, 1930, 33, 929.

TABLE I.
Calcification of Tubercles in Lungs of Animals Receiving Daily Injections of Calcium Chloride.

Non-injected Animals			Injected Animals			
Rabbit No.	Interval between injection of tubercle bacilli and death of animal	Number of caseous areas with calcium deposits per section	Rabbit No.	Interval between injection of tubercle bacilli and death of animal	Total amount of 5% CaCl ₂ injected	Number of caseous areas with calcium deposits per section
	days			days	cc.	
1	26	0	8	27	32	3
2	28	1	9	28	32	2
3*	31	0	10	34	55	4
4	33	0	11	34	60	2
5	34	1	12*	36	19	7
6	34	1	13	43	19	7
7	37	0				
Average		0.43				4.1

* These 2 animals were not killed but allowed to die of tuberculosis.

than in the few non-injected rabbits, each of which showed 1 spontaneous calcified tubercle.

Further experiments are being conducted to substantiate these preliminary results by quantitative analyses and also to determine the effect of the accumulation of calcium in tuberculous areas on the course of development of the disease.

5644

Further Studies by the Angiostomy Method of Hormonal Secretion of Pancreas and Suprarenal.

E. S. LONDON AND N. P. KOTCHNEFF.

From the Department of General Pathology, State Institute of Experimental Medicine, Leningrad.

These experiments are concerned with the study of the effect of the administration of various substances upon the function of the pancreas and the adrenal.

The method employed consisted of the use of an angiostomy cannula implanted on the superior pancreatico-duodenal vein and the suprarenal vein. The use of this cannula had already been recorded.

Procedure. Three control samples of blood were drawn from the angiostomized vessels and from the femoral artery. 0.2 cc. of each