female excretes 30 to 100 international units of androgen daily.[8, 9] The known androgens in female urine and their relative amounts are in a proportion of 1.3 mg androsterone to 0.2 mg dehydroandrosterone.[10] An international unit of androsterone is 0.1 mg and of dehydroandrosterone is 0.3 mg. With the maximum excretion of 100 international units this would be equivalent to approximately 8.7 mg androsterone and 4.0 mg of dehydroandrosterone. These urinary androgens, unless carefully separated from the estrogenic fraction, could then be sufficient to interfere seriously with the accuracy of an assay by the Astwood method in the non-pregnant female.

11574 P

Effect of Crude Peanut Oil Extracts of Brown Fat on Metabolism of White Rat.

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This paper presents preliminary data on the effect of the intraperitoneal injection of crude peanut oil extracts of the brown fat of the woodchuck and the thirteen-lined ground squirrel on the heat production of the white rat.

The earlier literature on brown fat has been reviewed by Rasmussen.[1] Various theories as to function have been proposed, but there has been a paucity of chemical and biological studies. Brown fat has been noted in infants[2] and cretins.[3] More recent work has suggested a further relationship to metabolism.[4, 5, 6] Wendt[7] reported

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10 Callow, N. H., and Callow, R. K., *Biochem. J.*, 1939, **33**, 931.

1 Rasmussen, A. T., *Endocrinology*, 1922, **6**, 760.

2 Hatai, *Anat. Anzeiger*, 1902, **21**, 369; Batty Shaw, *J. Anat. and Physiol.*, 1902, **36**, 1; Bonnot, *J. Anat. and Physiol.*, 1908, **43**.

3 Curling, T. B., *Med.-Chirurg. Transact. London*, 1850, **33**, 303; Hilton Fagge, *ibid.*, London, 1871, **54**, 154; Shattock, *Proc. Roy. Soc. Med.*, 2, Path. Sect., 1909, 252.

4 Vignes, H., *Compt. Rend. des Sci. et Mem. de la Soc. Biol.*, 1913, **75**, 360.

5 Felix, K., u. Eger, W., *Deutsches Arch. f. klin. Med.*, 1938, **182**, 41.

6 Friedman u. Feinschmidt, *Charkow. Erg. d. Bio.*, 1932, **8**.

7 Wendt, C. F., *Z. f. Phys. Chemie*, 1937, **249**, IV.

that injection of extracts of brown fat of hibernating hedgehogs into rats caused a lowering of the metabolism from 20-30% in 2-3 hours as measured by the Zeiss laboratory interferometer (method of preparation of extract not given). Control injections of olive oil did not cause this effect.

The experiments here reported were performed from April, 1939, to March, 1940. Hibernation was induced by first dehydrating the animals and then placing them in the cold (36°F-40°F) for 3 weeks to 3 months without food or water. The ground squirrels were killed by decapitation and the woodchucks by intracardiac air injection. Only axillary brown fat was used. The tissue was removed rapidly, immediately frozen in liquid nitrogen and later crushed in a tissue crusher.[8] The crushed tissue was then thoroughly ground in a mortar with peanut oil (approximately 5 cc of the latter per gram of tissue). After filtration of the supernatant fluid 1 cc of the filtrate was injected intraperitoneally into white, litter-mate rats. The rats were fed standard laboratory rations. The heat production was calculated from the oxygen consumption measured in an apparatus described elsewhere by Teague[9] and by Swann and Johnson.[10] Each rat was trained daily in the apparatus for 2 weeks before heat production studies were made and only well-trained rats were used.

The results are given in Table I. In the initial experiments during April and May, 1939, (Nos. 1, 2, 3) it was noted that injection of peanut oil alone elevated the heat production, whereas injection of crude peanut oil extracts of brown fat lowered it. Because of these results further experiments were performed during May and June, 1939. These failed to show any decrease in oxygen consumption within 3 hours after injection of the crude extract of brown fat. However, in several rats where the determination was made 2 days after injection (Nos. 4, 5, 6), there was, at that time, a marked depression of heat production. Because of this variability more detailed studies were made during July, 1939, (No. 7) which showed a definite fall in metabolism 1 and 2 days after injection. Controls did not show any changes. The injection of a similarly prepared crude extract into an hypophysectomized rat (No. 8) was followed by a sharp fall in heat production (35% in 3 hours) and the animal died 12 hours after injection (no gross findings at autopsy). The same crude extract caused a slight lowering of oxygen consumption in a normal rat (No. 9), whereas peanut oil alone caused a slight elevation (No. 10).

[8] Graeser, J. B., Ginsberg, J. E., and Friedemann, T. E., *J. Biol. Chem.*, 1934, **104**, 149.

[9] Teague, R. S., *Endocrinology*, 1939, **25**, 953.

[10] Swann, H. G., and Johnson, P. E., *Endocrinology*, 1939, **24**, 397.

TABLE I.

Comparison of Effects of Intraperitoneal Injection of Peanut Oil and of Crude Peanut Oil Extracts of Brown Fat of Woodchucks and Ground Squirrels on Heat Production of the White Rat.

Rat No.	Date 1939	Wt, g	Control	Injections 1 cc	Heat production Calories per square meter of body surface* per 24 hr: Hr after injection 1	2	3	Days after injection 1	2	3	5	6
1	4-20	186	715	P.O.[1]	700	1001	912		720			
	4-22	196	720	"	790	841	892					
	5-2	196	732	B.F.[2]	470	470						
2	4-20	162	895	P.O.	910	1031	1175		901			
	4-22	170	901	"		1131	1120					
	5-2	174	875	B.F.	579		761					
3	4-20	156	750	P.O.		1130	894		785			
	4-22	160	785	"	845	826						
	5-2	164	800	B.F.	615	724						
4	6-18	252	820	B.F.		826			615			
	6-27	268	1080	"		970			680			770
5	6-27	272	805	"	809	785			666			785
6	6-18	250	799	"	850				591		715	846
7	7-21	280	969	"	870	972		695	575	790	790	815
8†	6-6	190	625	"	560	442	405	Died 12 hr after inj.				
9	6-6	170	940	"	870	897	940					
10	6-6 1940	260	715	P.O.	765	832	841					
11	3-16	225	1060	B.F.	725	800		846	755	970	980	
12	3-11	0.5 mg thyroxin intraperitoneally daily										
	3-16	190	1600	B.F.	1131	1058		1190	1690	1400		1410
	3-25	205	1410	P.O.	1230	1230		1715				
13	3-11	0.5 mg thyroxin intraperitoneally daily										
	3-18	191	1300	P.O.	1510	1510		1260				

*Surface area calculated from Rubner's formula $9.1\sqrt[3]{wt^2}$. †Hypophysectomized rat. [1]P.O.—Peanut oil. [2]B.F.—Brown fat.

The effects of similarly prepared extracts of omental fat and kidney were studied in 4 experiments. In no instance was a lowering of the oxygen consumption noted after injection. During March, 1940, further determinations were again made and the effects of crude extracts were studied in 1 normal (No. 11) and 2 thyroidized rats (Nos. 12, 13).

Summary. It would appear that in these experiments the intraperitoneal injection of crude peanut oil extracts of the brown fat of the woodchuck and the thirteen-lined ground squirrel produced a lowering of the heat production of the white rat either at once or after 1 or 2 days (as measured by the oxygen consumption). Such effects, with one exception, were not produced by the injection of peanut oil alone.

11575

Protection of Guinea Pigs Against Mexican Typhus Virus by Vaccine from Infected Rat Lungs (Castaneda).

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In recent years much experimental work has been done on the production of a vaccine effective against typhus. The principal effort has been to devise a method that yields rickettsiae in sufficient quantity to be of practical application to the prevention of this disease. Since the use of infected lice as a source of rickettsiae, as developed by Weigl in 1930, the most promising methods have utilized peritoneal washings of infected white rats previously treated with benzol or irradiated with X-rays (Zinsser and Castaneda[1]); infected tissue cultures on agar slants (Zinsser, FitzPatrick and Wei[2]); material from the infected yolk sac of the developing chick (Cox, and Cox and Bell[3]); and a combination of the yolk sac and agar tissue culture methods (Zinsser, Plotz and Enders[4]). Castaneda has recently reported the production of an experimental typhus pneumonia in white

1 Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1930, **52**, 649; PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 840.

2 Zinsser, H., FitzPatrick, F., and Wei, H., *J. Exp. Med.*, 1939, **69**, 179.

3 Cox, H. R., *U. S. Pub. Health Rep.*, 1938, **53**, 2241; Cox, H. R., and Bell, E. J., *U. S. Pub. Health Rep.*, 1940, **55**, 110.

4 Zinsser, H., Plotz, H., and Enders, J. F., *Science*, 1940, **91**, 51.