

TABLE I.

Period	Time in min	Urine flow cc/min	Diod. Iodine mg %		Inulin mg %		Plasma Diodrast Clear	Plasma Inulin Clear
			Plasma	Urine	Plasma	Urine		
Patient RD								
I	31	10.7	3.6	82.8	22	127	258	65
II	19	7.6	3.88	131	28.8	230	268	64
III	30	4.27	3.57	186.4	30	384	234	57
Patient GW								
I	27	5.11	3.28	204	16	227.5	296	68
II	30	7.53	3.6	145.2	18.2	184	283	71
III	31	7.36	3.3	124.2	19.3	202	258	72
Patient MC								
I	30	2.74	2.13	316	14	450	463	100
II	23	3.87	1.94	198	17	397.6	450	103
III	16	6.88	1.82	95	19.9	270	408	106

and inulin are not due to defective renal function; curves on normal subjects are essentially similar. All of the urine specimens were voided and the patients were more or less ambulatory throughout the observation periods. The plasma values are interpolated to the mid-period from actual determinations made before the beginning of Period I, during Period II, and after the end of Period III. Clearances are in terms of cc/min/1.73 sq meters.

Patient RD was also examined by the intravenous technic, the urine being collected by bladder washings through a catheter. Consecutive clearances were as follows: diodrast (274, 264, 270); inulin (68, 66, 74).

Summary. Satisfactory plasma clearances of diodrast and inulin can be obtained in humans following subcutaneous injection of the respective substances.

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Reproduction in Rats on Synthetic B-Complex Supplement.*

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Several members of the vitamin B complex are now available in synthetic form. In a search for unidentified nutritional factors, the following diet (diet R-1) was fed to black rats: sucrose, 60 g;

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casein, acid-washed, 25 g; hydrogenated cottonseed oil ("Crisco"), 8 g; salt mixture,¹ 5 g; fish oil blend (3000-A, 400-D), 0.3 g; choline chloride, 50 mg; pantothenic acid, 2.8 mg;† nicotinic acid, 1.0 mg; riboflavin, 0.5 mg; thiamin, 0.2 mg; pyridoxine, 0.2 mg.‡ In addition, the rats received a weekly dose of 0.5 cc of corn oil mixed with 0.05 cc of shark liver oil containing 200,000 units of vitamin A per gram. In this diet, the fat-soluble factors are fed in a crude form, but the water-soluble factors are all synthetic and are fed at levels which were thought to be more than sufficient in each case. Nicotinic acid is not known to be needed by rats, but was fed as a precautionary measure. Growth on diet R-1 took place at about 60% of the normal rate for the strain for the first month, but at 80 days the rats had attained about 80% of normal weight.

In the first experiment 4 female rats were placed on diet R-1 at weaning (19 days of age). After 76 days they were mated to males which had been reared on a stock diet. Three litters of 8, 6, and 6 offspring respectively were produced and were continued on diet R-1. Seven rats now survive from these litters and averaged 160 g in weight at 80 days. Six of the rats appeared normal and had black hair. The seventh, a male, was slightly grey. The young in this and the succeeding experiment were about 60% of normal weight at birth.

In the second experiment, 2 groups of female rats were placed on diet R-1 at weaning and at an age of 113 to 153 days were mated with males which had also been on diet R-1 since weaning. One cc of wheat germ oil was fed to each female after mating. The first group consisted of 6 females and was continued on diet R-1 through pregnancy. Five litters, totalling 37 young, were produced. Three of these litters, totalling 22 young, survived and appeared to be developing normally at one week of age. The second group consisted of 9 females and was placed at mating on a diet which contained no pantothenic acid, but otherwise was identical with R-1. The 3 females which had produced litters in the first experiment were included in this group. Three rats in this group resorbed, and 5 of the others produced litters, totalling 28 young. The young were apparently healthy, but suckling was never observed, and they all died within 4 days of birth.

The feces of the rats on diet R-1 were small and black, which did

¹ Jukes, T. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **42**, 180.

† Prepared by Dr. S. H. Babcock.

‡ 2-methyl 1,4-naphthoquinone diacetate, 0.04 mg, has recently been included. This addition was made after an entire litter of "second generation" rats bled to death upon being earpunched at the age of 3 weeks. No tendency to prolonged bleeding has been observed since vitamin K has been added to the diet.

not indicate refection. Greying has not been observed in the female rats, but 5 males, averaging 394 g in weight, showed definite greying at 163 days of age after being kept on the diet since weaning. The high mortality of the young may have been due partly to injuries received from the wire floors during the first few days. Diet R-1 permits some degree of reproductive success, but is obviously not as good as a "normal" diet of natural foods. It is of interest, however, to record the rearing of healthy representatives of a second generation of rats on a diet supplemented with the B-vitamins in a synthetic form.

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**Purification of Follicle-Stimulating Hormone (FSH)
of the Anterior Pituitary.***

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Purification of the FSH has been achieved by the following steps: (1) extraction of acetone desiccated sheep pituitaries with 40% alcohol; (2) precipitation from 50% acetone at pH 4.5; (3) ammonium sulphate (A.S.) fractionation in which the hormone is reprecipitated several times between 0.5 and 0.67 SAS; (4) precipitation from 33% acetone at pH 4.8; (5) precipitation from 67% alcohol; (6) removal of less active material by saturation with NaCl at pH 4.1.

Details of this procedure have been published.¹ The product contained 1 unit in 6 γ (for method of assay and definition of unit, see below). It has recently been found that fractionation of very dilute FSH solutions (0.075%) with A.S. generally leads to doubling of hormonal activity in a fraction precipitating between 0.6 and 0.7 saturation with A.S. (the protein concentration at this stage being

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¹ Fraenkel-Conrat, H., Simpson, M. E., and Evans, H. M., *Anales de la facultad de Medicina, Montevideo*, 1940, XXV.