The addition of 0.3% of *dl*-tryptophane to Rations R-1c and R-1c + linseed oil meal extract stimulates growth and possibly increases the hemoglobin level (Table II) although it does not seem to duplicate completely the effect of 25% of linseed oil meal residue plus extract. In this connection it is interesting to point out that Woolley and Sprince[7] have shown that the dietary essential for guinea pigs designated at GPF 2 and found in linseed oil meal residue can be replaced by a mixture of cellulose and casein or cellulose, arginine, cystine and glycine. Apparently the guinea pig is susceptible to certain amino acid deficiencies and the limiting amino acid is dependent upon the kind of ration used. Since we have obtained excellent growth and development on a stock ration containing only 17% total protein it is evident that the amino acid requirement of guinea pigs is easily influenced by the remaining components of the diet.

Linseed oil meal extract does not seem to potentiate the action of tryptophane, nor in this series did it increase the growth stimulus supplied by 25% of linseed oil meal residue. Although growth on the linseed oil meal supplemented rations was much poorer than in the series reported in Table I, it should be noted that the addition of linseed oil meal does allow growth almost equal to that of animals from the same source receiving stock ration.

Summary. Guinea pigs fail to grow on a corn-soybean oil meal-alfalfa ration even when adequate amounts of vitamins A, D, E, and C are supplied. The survival and blood picture of the animals are improved by the addition of a mixture of crystalline water soluble vitamins of which niacin appears to be an important, but not the only, active component. Linseed oil meal, soybean oil meal, and casein supplements promote growth and blood formation approaching that of animals on stock ration. The potency of these substances is proportional to their tryptophane content and may be replaced in part by pure tryptophane.

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15620 P

Effects of Androgen upon Reproductive Organs of Normal and Castrated Fetuses with Note on Adrenalectomy.*

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This report deals with progress of studies designed to determine whether in placental mammals the normal, prenatal development of the reproductive organs is influenced by hormones. Recently it has been found that in unborn rats the number and size of the interstitial cells of Leydig may be increased experimentally by injecting gonadotrophin under the skin of the unborn.[1]

In the present experiments the animals used were rats in which pregnancy was dated from the time of observation of copulation. Fetuses were killed at about one hour preceding the estimated time of normal parturition (21 days and 16 hours after copulation). After weighing a fetus, its pelvic region was fixed in Bouin's solution and sectioned serially at 10 μ. The sections were stained with hematoxylin and eosin or with

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1 Wells, L. J., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 250.

TABLE I.
Volume of Reproductive Glands per Unit of Body Weight.*

Fetuses			Glands	
Groups	Treatment	Autopsy, hr after operation	Seminal vesicle, (mm^3/g) × 100	Bulbo-urethral, (mm^3/g) × 1000
A	None (controls)		.18 (18)	.27 (20)
B	Androgen (given to mothers)		.24 (11)	.45 (11)
C	Castration (Method 1)	20-61	.10 (6)	.17 (5)
D	Castr. (Method 1) and adrenalectomy	36-51	.09 (5)	.14 (5)
E	Adrenalectomy	51-61	.19 (3)	.28 (3)
	Fetuses of 6 litters.			
F	None (controls)		.17 (5)	.26 (5)
G	Castration (Method 2)	51-52	.11 (6)	.18 (6)
H	Castr. (Method 2) and androgen (pellet)	51-52	.21 (5)	.29 (5)
I	"Castration" (Method 3)	51	.12 (1)	.18 (1)

* Vertical columns in parentheses show number of fetuses from which the data were obtained.

alum cochineal and orange G. Volumetric determinations were made by paper-weight method of Hammar,[2] using the formula of Boyden.[3] All sections of a gland were projected at a magnification of 200 diameters. The epithelium of each section was drawn by outlining the basement membrane. In Groups A to E (Table I) the "volume of the seminal vesicle" is actually that of the gland, of the ejaculatory duct and of the proximal portion of the ductus deferens (portion to point where right and left tubes begin to diverge). In Groups F to I it is the volume of the gland and of the ejaculatory duct.

To investigate the effects of androgen upon the reproductive organs of normal male fetuses, 13 mothers were given subcutaneous injections of testosterone propionate.‡ Injections were made daily on days 13 to 22 of pregnancy, the daily dose being 15, 10 or 5 mg (Group B). It was found that at autopsy the fetuses of injected mothers were smaller than those of controls. The data suggest that the androgen had stimulated growth of the seminal vesicles and the bulbo-urethral glands. Although Greene, Burrill and Ivy failed to note any effects of androgen upon the reproductive organs of male fetuses, their studies did not include volumetric determinations.[4]

In order to determine whether the accessory reproductive organs are influenced by hormones produced by certain fetal glands, fetuses were subjected to laparotomy[5] and deprived of their testes by severing the *gubernaculum testis* and the *ductus deferens* (inguinal incisions, Groups C and D). Adrenals were removed through oblique lumbar incisions (Groups D and E). Since the volume of the test organs of Groups C and D was much smaller than that of controls, the question arises as to whether this was due to surgical alteration of the blood supply of these organs incidental to castration.

That this was not the case is suggested by the data on test organs of fetuses castrated by carefully shelling the testes out of the epididymides (Method 2, Group G). Similarly, it is suggested by the data obtained from a fetus "castrated" by shelling out the contents of the tunica albuginea (Method 3, Group I).

That the accessory reproductive organs of castrated fetuses were small because they had been deprived of a hormone produced by the testes (androgen) is suggested by the data on castrated fetuses in which, at the time of castration, a pellet of testosterone propionate

[2] Hammar, J. A., *Z. f. angew. Anat. u. Konstitutionslehre*, 1914, **1**, 312.

[3] Boyden, E. A., *Carnegie Inst. Wash., Contrib. to Embryol.*, 1940, **28**, 157.

‡ Perandren, supplied by Ciba Pharmaceutical Products, Inc., through the courtesy of Ernst Oppenheimer, M.D.

[4] Greene, R. R., Burrill, M. W., and Ivy, A. C., *J. Exp. Zool.*, 1941, **87**, 211.

[5] Wells, L. J., *Anat. Rec.*, 1946, **94**, 530.

was implanted under the skin of the back (about 1 mg). Hormone from the pellet could reach the test organs only provided they still had an adequate blood supply.

Preliminary data on the number of mitoses in transverse sections of the seminal vesicle were obtained by counting the mitotic figures in the last 10 sections preceding junction of seminal vesicle and ductus deferens to produce ejaculatory duct. In Groups F to I the averages were, respectively, 20.5, 8.1, 17.0 and 12.0.

Pending additional data, it would seem that in this species the fetal testes produce a hormone which accelerates the growth of such organs as the seminal vesicles and the bulbourethral glands. If the adrenals of fetal males produce any such hormone, the quantity is not sufficient to prevent the effects of castration upon the accessory reproductive organs.

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Osmotic and Mechanical Fragilities of Erythrocytes of Monkeys Infected with *P. knowlesi* Malaria.*

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The objective of the experiments reported here was (a) to determine the osmotic and mechanical fragilities of the red blood cells of monkeys infected with malaria and (b) to compare the osmotic fragilities of the red blood cells before and after trauma.

Methods. Samples of the freshly drawn oxalated blood of 7 *Macaca mulatta* monkeys experimentally infected with *P. knowlesi* 5 to 9 days previously and of 6 uninfected control monkeys were studied.† For the determination of the osmotic fragility of the red blood cells, 0.1 cc of blood was introduced into 1 cc of a series of progressively hypotonic concentrations of NaCl and into 1 cc of distilled water. Approximately 10 minutes later these samples were centrifugalized at 1500 r.p.m. for 10 minutes. The percentage concentration of hemoglobin in each supernatant was then determined by appropriate use of the Evelyn photoelectric colorimeter, taking that in the tube containing distilled water as a concentration of 100%. A smooth curve was then drawn connecting the points representing the percentages of hemoglobin liberated at each tonicity. From this were read off the respective concentrations of NaCl required to liberate the characteristic percentages of hemoglobin shown in Table I.

The mechanical fragility of the red blood cells was determined according to a recently published method.[1] One-half cc samples of blood with hematocrits adjusted to about 40% were traumatized by glass beads rolling in the greatest internal circumference of 50 cc rubber-stoppered Erlenmeyer flasks, rotated about a horizontal axis perpendicular to their flat base. The percentages of hemoglobin liberated by 1½ hours of such trauma were considered to represent the mechanical fragility of the samples.

Usually an infected and a normal control sample were studied simultaneously. The initial osmotic and mechanical fragilities of a portion of each sample were determined. If there was sufficient blood, the osmotic

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† The blood samples were obtained through the kindness of Dr. Quentin M. Geiman from animals being studied under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[1] Shen, S. C., Castle, W. B., and Fleming, E. M., *Science*, 1944, **100**, 387.