ever, a definite and very long lag, and sometimes a complete failure to grow where the inoculum is light. A similar state of affairs appears to exist with the majority of freshly isolated strains which have been tested. They will grow with a comparatively heavy inoculum but not with a light one. Parallel streakings of the same material done in the same way on ordinary blood meat infusion agar give prompt growth, with single colonies developing over night. It is evident, therefore, that some essential factor for early growth, or for growth from small inocula, is absent from medium prepared according to the present formula.

This factor has been shown to be present in blood, where it would seem to be confined mostly to the serum. It will withstand a reasonable amount of autoclaving and of course may prove to be multiple. It has not been possible so far to show that it is identified either with Vitamin B 1 (thiamine), Vitamin B 2 (riboflavine), pantothenic acid, or mixtures of these substances. The nature of this factor is being investigated further, and the purpose of the present note is merely to indicate that the full story of the nutritional requirements of the diphtheria group of organisms is not yet completely cleared up.

10519

Absorption and Titration of Androgenic Hormone in Alcoholic and Oily Solutions Administered Percutaneously.

BERNHARD ZONDEK AND FELIX SULMAN.

From the Laboratory, Gynecological-Obstetrical Department, Rothschild-Hadassah Hospital, Jerusalem.

The percutaneous resorption of oestrone in oily solutions has been described.[1] Recently one of us[1] showed that the absorption through the skin of oestrogenic hormone in organic solvents is quantitatively fully equivalent to the subcutaneous absorption of oily solutions. As organic solvents, 96% alcohol, benzol, ether, benzene, acetone and many others may be used.

Ito, Hajazu and Kon[2] reporting similar experiments emphasized particularly the ready absorption of estrogenic hormone in 60% alcohol.

Our experiments with androgenic hormone were directed towards

1 Zondek, B., *Klin. Wschr.*, 1929, 2229; *Lancet*, 1938, 1107.

2 Masao Ito, Seizi Hajazu and Turuziro Kon, *Zbl. Gyn.*, 1937, **61**, 1094.

the detection of a method which, with the help of organic solvents, enabled us to determine amounts even smaller than was possible with the help of Fussgaenger's method.[3]

We attempted to determine minute amounts of androgenic hormone with the recovery test method using the technic described by Tschopp.[4] Male rats weighing 60-80 g were castrated and after 4 weeks subjected to treatment with androgenic hormone. For 10 days 3 groups, each consisting of 5 animals, received 27 gamma of androgenic hormone daily. In Group 1 the animals received the hormone dissolved in 0.1 cc of olive oil subcutaneously. In Group 2 the hormone was administered percutaneously dissolved in one drop of olive oil, and in Group 3 the percutaneous method was also used, but the solvent consisted of 1 drop of 96% alcohol. On the 11th day the animals were killed, the genital organs placed in 4% formalin (1:10) and weighed 24 hours later. Table I shows the average weights given in milligrams after treatment with a total of 270 gamma of testosterone.

Table I shows that the amount of 270 gamma of testosterone administered daily for a 10-day period is ineffective if given percutaneously in oily solution, that it causes an increase in weight in the male sexual organs of 220% if given subcutaneously in oily solution and a weight increase of 50% if given percutaneously in 96% alcohol. Similar results were achieved with testosterone acetate.

It is evident that the percutaneous administration of androgenic hormone in alcoholic solution is, indeed, superior to the percutaneous use of an oily solution, its effectiveness being, however, only about a quarter of that achieved by the subcutaneous administration of an oily solution.

In searching for an even more sensitive test animal than the rat, we decided to use the baby chick comb test described by Burrows, Byerly and Evans[5] and confirmed by Frank and Klempner[6] and by Frank, Klempner and Hollander.[7] This method was recommended by Danby[8] as well. We used 6-day-old chicks in groups of 10 respectively. The animals received over a 10-day period androgenic hormone in oily solution subcutaneously or intramuscularly, in other

[3] Fussgaenger, R., *Medizin und Chemie*, I. G. Farben, 1934, **2**, 194.

[4] Tschopp, E., *Arch. Internat. Pharmacodyn. et Thérap.*, 1936, **52**, 381.

[5] Burrows, W. H., Byerly, T. C., and Evans, E. I., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 50.

[6] Frank, R. T., and Klempner, E., *ibid.*, 1937, **36**, 763.

[7] Frank, R. T., Klempner, E., and Hollander, F., *ibid.*, 1938, **38**, 853.

[8] Danby, M., *Acta brev. Neerland*, 1938, **8**, 90.

TABLE I.
Weight of Genital Organs of ♂ Rats After 10 Days' Treatment with a Total of 270 Gamma Testosterone.

Solvent	Method of administration	Seminal Vesicle mg	Prostate mg	Cowper's gland mg	Preputial gland mg	Penis mg	Total mg	Proportion
Controls		15.3	34.7	38.7	35	71.3	195	1
Olive oil	percutaneously	14.3	31	35	49.7	69	199	1
" "	subcutaneously	76.6	145.7	182.7	79.3	149.7	634	3.2
Alcohol 96%	percutaneously	19.3	56.3	56	65	95.3	292	1.5

groups the combs were brushed with oily or alcoholic solutions. On the 11th day the animals were killed and the heads fixed for 24 hours in 4% formalin (1:10). Then the combs were dissected off and weighed. The average weight of the combs of the control animals (26 mg) was designed as 1, and the proportional enlargement was calculated from the average figures found in weighing the combs of the test animals. Table II shows the results of several series of experiments after treatment with testosterone.

TABLE II.
Weight of the Combs of Chicks After Treatment with Testosterone.

No.	Solvent	Method of administration	Dose of testosterone per day (gamma)	Total dosage of testosterone (gamma)	Wt of comb (avg mg)	Proportion (control = 1)
1	Controls	brushed (dry)	—	—	26	1
2	"	not treated	—	—	27	1
3	*Ol. arachidis*	subcutaneously	0.3	3	28	1
4	" "	intramuscularly	8	80	38	1.4
5	" "	"	16	160	57	2.1
6	" "	"	32	320	123	4.5
7	" "	percutaneously	0.15	1.5	27	1
8	" "	"	0.3	3	44	1.6
9	" "	"	0.6	6	52	2
10	96% alcohol	"	0.05	0.5	42	1.6
11	" "	"	0.1	1	47	1.8
12	" "	"	0.15	1.5	53	2
13	" "	"	0.3	3	73	2.7

Table II shows that by brushing the combs of chicks with alcoholic testosterone solution, determinaton of even 0.5 gamma (10 x 0.05, see example 10) is possible at least. Considering the quantities of hormone necessary to obtain a weight increase of the comb of 100%, if the percutaneous brushing of the comb is used, it is evident that about 4 times as much hormone in oily solution (No. 9) must be used, as that necessary in the alcoholic solution (No. 12) in order to obtain similar results. Considering the amount necessary to increase the weight of the comb by 60% we see that the oily hormone solution (No. 8) must contain about 6 times as much of the hormone as does the solution in 96% alcohol. (No. 10.)

The above experiments demonstrate that the test method which we propose for the detection of androgenic hormone is about 5 times as sensitive as that of Fussgaenger,[3] who brushes the combs of chicks with oily solutions. Based on the proportions indicated by Fussgaenger we can more or less establish the scale of sensitivity in comb units for testosterone shown in Table III.

The beforementioned experiments were carried out on a total of

TABLE III.

Solvent	Method of administration	Site of administration	Dosage (or multipla)
96% alcohol	Percutaneously	Comb	1
Oil	,,	,,	5
,,	Subcutaneously	,,	12.5
,,	Intramuscularly	Glutaeus	250
,,	Subcutaneously	Back	875

130 chicks, 10 chicks making up one series of assay. The average values obtained with reference to the size of the combs always represented clearcut results. In other series, however, now and then cases were observed in which a single chick showed an increase of the comb of more than 100%, compared with the average value of the others within the same series. On principle, we abstained in such cases from using such unhomogeneous experimental series. In any case we advise also to use for the baby chick comb test at least 10 chicks per group.[5-8]

With reference to the weight of the combs in the 17-day-old control chicks—at the termination of the experiment—we noted considerable variations of the average values depending on the season (12 mg in winter as compared with 30 mg in summer).

Summary. Studying the percutaneous absorption of androgenic hormone (testosterone) it was noted that the hormone dissolved in alcoholic solutions is more readily absorbed than that in oily solutions. Most instructive is this difference in the comb test, because here the hormone can be applied at the very site of its effectiveness.

Using the baby chick comb test, we found that brushing the combs with alcoholic solutions of testosterone is about 5 times as effective as brushing the combs with oily solutions, according to Fussgaenger's method. The method proved to be much more sensitive than the subcutaneous and intramuscular administration of the hormone since it is possible to detect quantities as small as 10×0.05 gamma $= 0.5$ gamma of testosterone at least. We are recommending the standardization of small quantities of androgenic hormone with the use of this method.

If the recovery test is used, on castrated rats, the effectiveness of the percutaneous method using alcoholic solutions of the hormone is less marked. As a matter of fact, alcoholic testosterone solutions are more readily absorbed than oily solutions percutaneously; in this method, however, the subcutaneous administration of the hormone in oily solution is the most effective (4 times as sensitive as the alcoholic percutaneous method).