the cerebrospinal fluid 11 were sacrificed for histological study: 11 were subsequently given intracerebral or intranasal inoculations. Two of these were resistant to infection while the remaining 9 contracted typical poliomyelitis.

The last group is concerned with animals in which pial injury was produced either accidentally or deliberately. In the cortical drip experiments the dura was not sutured in 5 cases. Although the inoculum was reduced to 0.05 cc of 20% virus in 3 of these animals, paralysis resulted in each instance. At autopsy the chief finding was a cortical herniation which was either very hyperaemic or frankly necrotic. In 2 instances a suture needle injury to the cortex was followed by paralysis.

The idea that infection was made possible by a change in pial permeability is confirmed by the following experiment. In 3 animals 0.5 cc of a distilled water suspension of 20% MV virus was carefully introduced into the lumbar cistern by the technique already described. In each instance paralysis resulted although autopsy revealed no visible damage to the cord or nerve roots.

The protective barrier of the pia is thus conceived to be largely a mechanical one, and under the influence of factors which bring about changes in its permeability. Under normal conditions it is probably effective enough to render the cerebrospinal fluid of the subarachnoid space negligible as a virus-disseminating medium. These findings may be interpreted as contributing further evidence for the neurotropism of poliomyelitis virus. The resistance of the ventricular ependyma will be similarly considered in succeeding experiments.

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Penetration of Sesame Oil Painted on the Capon Comb

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Androgenic substances, extracted from human urine by a method previously reported,[1] were dissolved in sesame oil and applied to the surface of the combs of white leghorn capons. Since percutaneous

[1] McCahey, J. F., Hansen, L. P., and Soloway, D., *J. Urol.*, 1937, **38**, 397.

application of androgens, dispersed in oil or other fatty vehicle, has been used both for assay on the combs of chicks and capons, and as a method of therapy in man, it is not only of interest to know how effective the androgens are when so applied, but also what happens to the vehicle. The time required to induce growth and the actual increase in size of the comb, are measures of the rapidity and adequacy of penetration of the androgens through the various layers of the skin, while histological studies with special stains for fatty substances indicate the fate of the sesame oil.

The greater growth of the capon comb in response to surface applications of androgens, as compared with the growth obtained from parenteral administration has been noted.[2, 3, 4, 5] Table I shows the growth of a capon comb resulting from anointment with urinary androgens, compared with the response obtained previously in the same bird when 4 times the amount of the same material was injected. The total length of the comb and the height of each of its points were measured in millimeters for all birds used for several weeks before the applications and daily thereafter until the animals were sacrificed. Note that the response was from 2 to 3 times greater with anointment than it was with injections.

TABLE I.
(Capon No. 5.)
Comparison Between Growth Response of Capon Comb to Intramuscular Injections and Direct Application of Urinary Androgens.

Date	11/1	11/11	11/12	11/14	11/15	11/16	Max. growth, mm
Injection		1 cc	1 cc				
Total length, mm	57.5	58.0	61.0	67.0	67.0	67.0	9.0
Height of points, mm							
No. 1	26.5	28.0	29.0	35.5	35.0	34.5	7.5
2	26.5	27.5	28.0	33.0	32.5	33.5	6.0
3	25.8	27.0	27.0	32.3	32.5	33.0	6.0
4	22.3	22.5	24.0	28.8	28.0	29.0	6.5
Date	1/31	2/17	2/18	2/20	2/21	2/24	Max. growth, mm
Application		¼ cc	¼ cc				
Total length, mm	64.5	64.5	69.0	79.0	82.0	82.0	17.5
Height of points, mm							
No. 1	31.5	32.0	36.0	45.5	46.5	47.0	15.5
2	29.0	30.0	34.5	42.5	43.0	46.0	16.0
3	29.0	29.0	34.5	42.0	43.0	44.5	15.5
4	25.0	25.0	31.0	39.0	41.0	42.0	17.0

[2] Fussgänger, R., Medicine in Its Chemical Aspects, I. G. Farbenindustrie A. G., 1933, 1, 198; 1934, 2, 185.

[3] Soloway, D., Hansen, L. P., and McCahey, J. F., *Anat. Rec.*, 1936, **64**, 46.

[4] Dessau, F., and Freud, J., *Acta brev. Neerland. Physiol.*, 1936, **6**, 9.

[5] Deanesley, R., and Parkes, A. S., *Proc. Roy. Soc.*, B, 1937, **124**, 279.

The combs of 12 capons (which showed complete absence of testes at autopsy) were painted with ¼ cc of the sesame oil-containing extract representing the androgens recovered from 125 cc of male student urine. Small drops of the material, from a delicate syringe, were placed upon the surface of the comb and spread with a very fine camel's hair brush over the whole surface, avoiding pressure or rubbing. Thus a thin film of oil was placed upon the most superficial portion of the epidermis of the comb. One comb was removed 3 hours after the application, one at 6, one at 9, one at 12 hours after. The rest of the combs received another similar application 24 hours later after which one comb was removed at the end of each successive 24 hour period. Thus, various stages showing penetration of the oil as well as reaction within the comb to androgens were obtained within the limits of 3 to 188 hours.

Several combs were painted with sesame oil only, and numerous untreated, unstimulated capon combs were also sectioned.

Frozen sections of a piece of each comb were stained with Sudan III, Scharlach R and Cyanin. Other pieces were placed in various fixatives (formalin, Zenker's-formol, Bouin's, Hermann's, Champy's, etc.) and in aqueous solutions of Osmic acid. Sections from each piece were stained with hematoxylin and eosin, Mallory's, iron hematoxylin and mucin stains.

Results. Although definite growth of the comb, wattles and earlobes was evident within 24 hours after the first application, demonstrating the rapid penetration and stimulating activity of the hormones, the sections of the comb stained for the sesame oil showed no penetration beyond the stratum corneum. The stained oil stopped abruptly at the inner limit of this layer. The entire area of the stratum corneum was orange to scarlet with Sudan, blue with Cyanin, and varying shades of dark brown to black with Osmic acid (the color depending upon the amount of oil applied and the thickness of the sections). The penetration of the oil through this keratinized layer occurs within the first 3 hours following the initial application. No clear evidence of any oil in or between the rest of the epidermal cells or in the dermis could be demonstrated at any of the stages studied.

Much of the oil seemed to accumulate and persist in the spaces between the projecting surface papillae, and here, keratin-like granules within the most superficial cells adjoining the inner margin of the cornified layer occasionally showed a slight affinity for the Sudan stain. The white, thickened (extra-keratinized) layer, char-

acteristic of the capon comb, was rapidly made translucent by the oil so that the underlying epithelial cells and the capillaries in the dermis became fairly visible and the shrunken, closely approximated surface papillae more evident.

The same amount of penetration of the sesame oil, when used alone, was noted. The stained sections of untreated, unstimulated, unoiled combs suggest the presence of some substance in the stratum corneum that reacts slightly with Osmic acid, giving a brown color, but not the black of combs painted with the oily solution. This reaction may be due to cholesterol or keratin present in the horny layer.

Although the sesame oil could not be demonstrated, by the methods used, in or between the deeper cells of the epidermis or in the dermis, this does not remove the possibility that absorption of the oil may occur in an unstainable form. However, there is a marked color reaction still present in the cornified layer 188 hours after the first application, indicating the presence of most of the oil. Studies[6,7] on the absorption through the human skin indicate that the type of oil represented by sesame does not penetrate the barrier of the skin. Besides, there are no gland openings or hair follicles to aid absorption in the comb, and although there are some feathers near the base of the comb, care was exercised to avoid painting them.

Removal of sesame and similar oils injected into connective tissue and muscle, or used as vehicles for hormones, has been investigated[8, 9, 10, 11, 12, 13] with the conclusion that they undergo a very slow absorption and penetration even within these internal structures. Similarly, studies of absorption of such oils from mucous membranes also indicate comparable behavior.[14, 15]

The urinary androgens are probably slowly partitioned from the oil at the inner border of the stratum corneum and then selectively absorbed through the rest of the epithelial cells into the dermis and its capillaries. The ability of crude urinary extracts of androgens to penetrate the epidermis and spread through the dermis may rep-

6 Sollmann, T., *A Manual of Pharmacology*, 1927, 3rd ed., p. 76.

7 Macht, D. I., *J. A. M. A.*, 1938, **110**, 409.

8 Korenchevsky, V., Dennison, M., and Schalit, R., *Biochem. J.*, 1932, **26**, 1306.

9 Korenchevsky, V., Dennison, M., and Kohn-Speyer, A., *Biochem. J.*, 1933, **27**, 778.

10 Deanesly, R., and Parkes, A. S., *J. Physiol.*, 1933, **78**, 155.

11 Bülbring, E., and Burn, J. H., *J. Physiol.*, 1935, **85**, 320.

12 Deanesly, R., and Parkes, A. S., *Lancet*, 1936, 230, **1**, 837.

13 Parkes, A. S., *Brit. Med. J.*, 1938, **1**, 371.

14 Eleftheriou, D. S., *Compt. rend. Soc. Biol.*, 1936, **123**, 231.

15 Eleftheriou, D. S., *Compt. rend. Soc. Biol.*, 1936, **123**, 1186.

resent a property similar to the spreading effect (R-factor) first obtained by Duran-Reynals[16] and others from testis tissue extracts injected intradermally. McClean[17] described the histology of an area of the dermis of the rabbit similarly injected, wherein the collagenous bundles were thought to separate into smaller divisions and fibrillae, resembling very early changes noted in the tissues of the dermis of the capon comb under influence of androgens.

Conclusions. (1) Sesame oil is not absorbed (at least within 188 hours) beyond the stratum corneum of the epidermis of the capon comb, following surface application of the oil alone or the oil acting as a vehicle for androgens. (2) Urinary androgens are selectively absorbed from sesame oil at the inner margin of the stratum corneum, then rapidly penetrate to induce characteristic growth changes. (3) The ability of crude urinary extracts used in these experiments to penetrate the barrier of the epidermis and spread in the dermal and subdermal tissues is similar to the spreading effects of intradermally injected testicular extracts. (4) The greater growth reaction in the comb due to percutaneous applications as compared with parenteral administration of the same androgenic material, appears to be a matter of rate of absorption more nearly approaching the normal physiological requirements of the bird. This involves a gradual and adequate separation of the hormone from the oil, a rapid penetration of the rest of the epidermis and dermis and a large dermal network of capillaries, present even in the capon crest.

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[16] Duran-Reynals, F., *J. Exp. Med.*, 1929, **50**, 327.

[17] McClean, D., *J. Path. and Bact.*, 1931, **34**, 459.