mal but more slowly than in the intact animal(4).

Comparing the anabolic effects of the various agents in the dose employed in this study, it will be noted that esterification of testosterone with cyclopentylpropionic acid (Fig. 1) increases and prolongs anabolic activity to the greatest degree. With this agent (TCP) the anabolic effect is still maximal a month after injection. Aluminum phosphate delays both the measured actions of testosterone (Fig. 1 and 3) but does not significantly potentiate or prolong either effect of this agent. In the case of MASD (Fig. 2) there is no significant difference between the curves obtained with and without aluminum phosphate, probably because the steroid is highly insoluble. All the agents employed in this assay produce a great increase in the size of the levator ani muscle.

Androgenic activity is manifested by all these agents in the very large dose used. Aluminum phosphate does not delay, potentiate, or prolong the androgenic action of MASD (Fig. 4), presumably because of the solubility properties of the agent itself. Aluminum phosphate delays and somewhat prolongs the androgenic action of testosterone (Fig. 3); however, no great potentiation, *i.e.* increased efficacy, of this suspension is observed. Testosterone cyclopentylpropionate is the most potent agent in this group (Fig. 3). Its increased activity cannot be explained on the basis of slower release of the active constituent from the injection site alone, since there is no greater delay in growth of the levator ani muscle and seminal vesicles with the cyclopentylpropionic acid ester than with the aluminum phosphate suspension of testosterone. Inasmuch as the increased efficacy of this ester cannot be accounted for on the basis of delayed absorption, it seems likely that the long side chain delays the inactivation of the compound. This conclusion is in harmony with that of Miescher *et al.*(7) that the longer the carbon side chain, the more protracted is the effect. However, TCP differs from the esters studied by Miescher *et al.* in that prolongation of effect is not combined with decreased intensity of effect.

Caution must be exercised in transferring these observations to other species since, as Miescher *et al.* have pointed out, rats are more sensitive to the action of testosterone esters than are capons.

Summary. 1. Testosterone cyclopentylpropionate shows the greatest anabolic and androgenic efficacy of the agents studied. Its increased efficacy cannot be explained on the basis of delayed absorption, suggesting a delayed inactivation of this ester. 2. Aluminum phosphate delays the onset and decline of the anabolic and androgenic actions of testosterone in aqueous suspensions, but produces no such effect upon the actions of methylandrostenediol.

7. Miescher, K., Wettstein, A., and Tschopp, E., *Biochem. J.*, 1936, v30, 1977.

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Purification and Identification of the Antistiffness Factor.* (18506)

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In a previous study(1) evidence was presented that the infra-red absorption spectrum of the antistiffness factor obtained from sugar cane resembled the spectrum of stigmasterol more closely than any other spectrum of a

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1. Rosenkrantz, H., Milhorat, A., Farber, M., and Milman, A., *Fed. Proc.*, 1951, in press.

large series of steroid compounds investigated. Purification and characterization of the natural antistiffness factor in the earlier work was accomplished by solvent partition and spectrophotometric analysis. The present paper is a continuation of this work.

Counter-current distribution was performed in a 24-plate Craig apparatus(2), a methanol-isooctane two-phase system being utilized(3). Solvent partition was applied to a purified concentrate† from sugar cane, the migration of the material being followed by dry weight determinations and ultra-violet absorption characteristics. On the basis of both the latter analytical technics, the concentrate was divided into 3 fractions, A, B, and C. Infra-red analysis of each fraction ascertained the relative purity and aided in characterization of each fraction.

The infra-red absorption data indicated that fractions A and C were free of each other and that fraction A probably was a sterol. The sterol nature of fraction A was further demonstrated by a positive Liebermann-Burchard reaction. Since fraction A gave also a mild, positive Rosenheim color reaction, the presence of some diene impurity was suggested and this was supported by the ultra-violet absorption spectrum of fraction A, which at high concentrations was nearly identical to those of ergosterol, 7-dehydrocholesterol and other $\Delta^{5,7}$-diene steroids(4). It was not concluded by Ross, van Wagtendonk and Wulzen(5), despite low extinction coefficients which they obtained, that the similar absorption spectrum of their antistiffness factor was due to a diene impurity.

The infra-red absorption spectrum of fraction A was utilized in screening a large series of steroid compounds, efforts being concentrated on stigmasterol and ergosterol derivatives of the dihydroergosterol type because of spectral similarities. The presence of a hydroxyl group was established by the 2.95 and 9.45 μ absorption bands (elemental analysis of the antistiffness factor by Ross, *et al.*(5) had demonstrated the presence of only one oxygen) and this hydroxyl group was assigned a β-configuration on the basis of digitonin precipitation. Infra-red absorption bands of moderate intensity near 6 μ in the spectrum of fraction A suggested either contamination by oxidation products, a conjugated double bond system (not in a benzene ring system) or a combination of both. The absorption bands between 9 and 11 μ strongly suggested that the infra-red spectrum of fraction A was closer to that of stigmasterol than to that of ergosterol derivatives, cholesterol derivatives, α-spinasterol or the steroidal hormones.

Sublimation of fraction B, a mixture of fractions A and C which contained most of the distributed material, resulted in further purification. The infra-red absorption spectrum of this sublimate was nearly identical to that of stigmasterol but the material contained some fraction C. A combination of absorption bands near 9.5, 9.8, 10.2, 10.3 and 10.4 μ were characteristic of stigmasterol and the sublimate but not of the other steroids studied. Absorption bands near 5.8, 13.7 and 13.9 μ indicated the presence of fraction C, the non-steroid component. A complete identification of the antistiffness factor from sugar cane as being stigmasterol was afforded upon receipt of a purified sterol isolated from sugar cane by the Armour Laboratories.† The infra-red absorption spectrum of this antistiffness sterol was identical with that of stigmasterol. Structural interpretations and the use of infra-red data in characterizing stigmasterol (the antistiffness factor) will be discussed in a subsequent paper.

After this work had been completed, we were informed that Drs. Emil Kaiser and

2. Craig, L. C., *J. Biol. Chem.*, 1944, v155, 519.

3. Ulick, S., and Milhorat, A. T., *Science*, 1949, v110, 531.

† We wish to express our gratitude to the Eli Lilly Company for donation of the purified concentrates from sugar cane and to the Armour Laboratories for a sample of their purified sterol.

4. Morton, R. A., The Application of Absorption Spectra to the Study of Vitamins, Hormones and Coenzymes, Adam Hilger, Ltd., London, 1942, Chap. II.

5. Ross, L. E., van Wagtendonk, W. J., and Wulzen, R., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 281.

6. Kaiser, E., and Wulzen, R., *Arch. Biochem.*, 1951, in press.

Rosalind Wulzen(6) on the basis of chemical and biological evidence other than infra-red spectroscopy, also concluded that the anti-stiffness factor obtained from sugar cane wax is stigmasterol.

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Effect of Fecal Bacterial Activity on Rectal Temperature of Man. (18507)

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Claude Bernard(1) called attention to the fact that the rectal temperature was one of the highest to be found in the body. This observation has been confirmed by other investigators(2). It seemed possible that this elevation in rectal temperature might be due, in part at least, to bacterial activity in the rectum and rectosigmoid. Bazett(3) suggested such a possibility. If this hypothesis were true, reduction of the intestinal bacteria, as by an orally administered, poorly absorbed sulfonamide, might be expected to result in a decrease in rectal temperature. Ravdin *et al.*(5) and others have shown that the technic of succinylsulfathiazole administration such as was employed here will result in a marked reduction in the number of organisms present in the feces.

Methods. Five normal young adult males served as subjects. Each subject received, in 4 divided daily doses, succinylsulfathiazole orally, 25 mg per kilo of body weight per 24 hours. The drug was given in this manner for 7 days. The subjects' diets and physical activity remained approximately constant for the periods before, during, and after the drug administration. With the subjects supine, rectal temperatures were recorded for 30 minutes preceding the noon meal and in a constant relationship to the subjects' physical activity and bowel movements. Rectal temperature determinations were made by copper-constantan thermocouples inserted 8 cm into the rectum. Measurements were made 8 hours before the drug administration was begun and were continued throughout the course of the drug. Temperatures were recorded at 2-minute intervals by a modified Brown automatic recording potentiometer whose accuracy was within 0.05°C. The temperatures of the final 6 minutes of the daily observation periods were averaged to obtain the temperature of the individual for any one day.

In some instances, smears from fecal specimens were obtained at the time of the temperature measurements. From these smears gross estimates of the number of fecal bacteria present were made in the manner described by Dearing and Heilman(4).

Results. Although the fecal bacterial content decreased markedly following the succinylsulfathiazole ingestion, no significant changes in rectal temperatures were seen (Tables I and II).

Discussion. It is apparent that exothermic reactions associated with the fermentation processes of microorganisms in the lower colon and rectum are of minor importance in modifying the level of the rectal temperature in man. Since the metabolism of this portion of the gastro-intestinal tract is considered to be quite low the obvious factor involved in maintaining the temperature of the organ must be the temperature of the perfusing blood. The chief arterial supply of the lower 12 cm of the large intestine is derived from mesenteric branches of the aorta, all of which are within the abdominal cavity. The venous

1. Bernard, C., Lecons sur la chaleur animale, Paris, 1876.

2. Horvath, S. M., Rubin, A., and Foltz, E., *Am. J. Physiol.*, 1950, v161, 316.

3. Bazett, H. C., personal communication.

4. Dearing, W. H., and Heilman, F. R., *Proc. Staff Meeting of the Mayo Clinic*, 1950, v25, 87.