terized further. The major activity resides in a cell-free supernatant of a phosphate buffer extract, *p*H 7.4, of rat liver ground in a meat grinder. The activity was retained in the supernatant of an acetone powder of the ground rat livers. Dialysis of this supernatant lowered the conversion of the 3-ketone to the 3α-hydroxy group to 30%, but with the addition of 0.002 *M* DPN, the conversion increased to 95%. Further studies of this reaction are in progress.

Experimental[6]

Hydrolysis of 21-Acetoxy-17α-hydroxypregnane-3,20-dione.—Three grams of 21-acetoxy-17α-hydroxypregnane-3,-20-dione was hydrolyzed with $KHCO_3$ in methanol at room temperature for 24 hours to yield 2.74 g. of white crystalline material, m.p. 185–195°. The material was chromatographed on a column containing 90 g. of silica gel and eluted with benzene, benzene–ethyl acetate mixtures and methanol. Elution with benzene ethyl acetate (2:1) yielded 2.3 g. of crystalline material which was recrystallized from acetone and ethanol to give crystals melting at 200–202°, $[\alpha]^{27}D$ +62° (ethanol).

Incubation of 17α,21-Dihydroxypregnane-3,20-dione (Dihydro S) with Rat Liver Homogenate.—The livers of adult male albino rats were quickly removed following decapitation and homogenized for 20 sec. in a Waring blender in 0.1 *M* phosphate buffer, *p*H 7.4. A total of 820 mg. of dihydro S was incubated (10 mg. of steroid per 10 g. equivalent of tissue wet weight) by shaking in air at 38° in a Warburg for 1.5 hours. Following acetone precipitation of the proteins, and a 70% methanol–petroleum ether partition to remove the lipids, the crude extract (2.77 g.) was chromatographed on a column containing 170 g. of silica gel.

Isolation of 3α,17α,21-Trihydroxypregnan-20-one.—The various fractions of the silica gel chromatogram were eluted with a system of benzene, benzene–ethyl acetate mixtures, ethyl acetate and methanol. Elution with benzene–ethyl acetate (2:1) yielded a total of 660 mg. of crystalline product. Aliquots of the various eluates were spot-checked by paper partition chromatography using the toluene–propylene glycol systems for four hours. The dihydro S and tetrahydro S zones were visualized with the triphenyltetrazolium chloride reagent.

Recrystallization of the pooled homogeneous fractions yielded 389 mg. of pure 3α,17α,21-trihydroxypregnan-20-one. The remainder of the crystalline material (271 mg.) proved to be a mixture of unchanged substrate and tetrahydro S isomers. 3β,17α,21-Trihydroxypregnan-20-one, m.p. 224–226°, was isolated from this mixture (2.0% yield).

3α,17α,21-Trihydroxypregnan-20-one was recrystallized from acetone and finally ethanol to give small platelets, m.p. 200–204°. Various melting points were obtained with different crystallization procedures, the highest melting crystals, obtained from ethanol, melting at 214–216°, $[\alpha]^{27}D$ +60° (ethanol). The diacetate melted at 201–206°, $[\alpha]^{27}D$ +77° (ethanol). A mixture with 3α,21-diacetoxy-17α-hydroxypregnan-20-one (m.p. 195–202°) melted at 195–204°.[7]

3α-Hydroxyetiocholan-17-one by Oxidation of 3α,21-Diacetoxy-17α-hydroxypregnan-20-one with Chromic Acid.—40.5 mg. of 3α,21-diacetoxy-17α-hydroxypregnan-20-one, m.p. 201–206°, was dissolved in 4 ml. of glacial acetic acid. To this was added 20 mg. of CrO_3 in 0.4 ml. of water and the solution was allowed to stand at room temperature for 3 hours. Excess CrO_3 was reduced, the mixture diluted with water to 50 ml., and extracted with ether exhaustively to give a crude crystalline product (30.3 mg.) melting at 80–90°. Following sodium hydroxide hydrolysis at 50° for 1 hour, 11.2 mg. of 3α-hydroxyetiocholan-17-one, m.p. 140, 150–151° was recrystallized from ether–petroleum ether.

3α-Hydroxyetiocholan-17-one by Oxidation of 3α,17α,21-Trihydroxypregnan-20-one with Sodium Bismuthate.[8]—Ten mg. of 3α,17α,21-trihydroxypregnan-20-one was dissolved in 1 ml. of glacial acetic acid, followed by the addition of 1 ml. of water to a 25-ml. erlenmeyer flask covered with aluminum foil. An excess of 50 mg. of sodium bismuthate was added and the flask shaken for 0.5 hour in a Warburg at room temperature. The mixture was diluted with water, filtered and thoroughly extracted with ether. The ether solution was washed once with 1 *N* sodium hydroxide, followed by water. The dried extract consisted of white crystalline material weighing 7 mg. (70%), m.p. 140°. Recrystallization from ether–petroleum ether gave needles of 3α-hydroxyetiocholan-17-one, m.p. 140, 150–151°.

3β-Hydroxyandrostan-17-one from 3β,17α,21-Trihydroxyallopregnan-20-one by Oxidation with Sodium Bismuthate.—26.3 mg. of 3β,17α,21-trihydroxyallopregnan-20-one was dissolved in 2 ml. of glacial acetic acid followed by the addition of 2 ml. of water in a 25-ml. erlenmeyer flask covered with aluminum foil. An excess of 100 mg. of solid sodium bismuthate was added and the flask shaken for 0.5 hour in a Warburg at room temperature. The extraction with ether as previously described yielded 23 mg. (87%) of a white crystalline product, m.p. 167–170°. Recrystallization from methanol yielded 3β-hydroxyandrostan-17-one, m.p. 172–176°.

An attempt to oxidize a 21-desoxy analog, 3β,17α-dihydroxyallopregnane-20-one, m.p. 242–249°, with sodium bismuthate was unsuccessful and yielded only the starting material.

(6) All melting points were taken on a Fisher–John apparatus and are uncorrected. Infrared analyses were carried out with a Perkin–Elmer model 12-C infrared spectrometer. The analyses and interpretations were carried out by Dr. H. Rosenkrantz and Mr. P. Skogstrom in our laboratories.

(7) B. A. Koechlin, T. H. Kritchevsky and T. F. Gallagher, THIS JOURNAL, **73**, 189 (1951), reported for 3α,21-diacetoxy-17α-hydroxypregnan-20-one, m.p. 205–206°, $[\alpha]D$ +88° (ethanol). A sample was generously supplied for comparison and proved to be identical as shown by infrared analysis.

(8) C. J. Brooks and J. K. Norymberski, *Chem. & Ind.*, 804 (1952).

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Observations on the Crystalline Forms of Galactose

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In an investigation[3] concerned with the examination of the hydrolyzate from a polysaccharide, it was necessary to recognize and identify the common crystalline forms of galactose. Five crystalline phases exist for this sugar. These are the racemate and the α- and β-pyranose forms for each enantiomorph. α-D-Galactopyranose is the stable form obtainable under the usual laboratory conditions. No record of the melting point of β-D-galactopyranose could be found. A sample of this material was prepared according to the directions of Hudson and Yanovsky.[4] It exhibited the mutarotatory properties reported by these authors and its X-ray powder diffraction diagram was that recorded by Werner[5] except that we found no evidence of contamination with the α-D-anomer. This preparation melted at 143–145°[6] when heated rapidly to near its melting point. On maintaining the temperature at 140°, the substance resolidified completely and then remelted at 164–167°. In some preparations an initial melting point at 147–150° was found and under continued slow heating, resolidification was noted at 152–155° with re-

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(3) M. L. Wolfrom, G. Sutherland and M. Schlamowitz, THIS JOURNAL, **74**, 4883 (1952).

(4) C. S. Hudson and E. Yanovsky, *ibid.*, **39**, 1013 (1917).

(5) I. Werner, *Microchemie ver. Microchim. Acta*, **39**, 133 (1952).

(6) All melting points are corrected and were observed in Pyrex glass.

melting at 164–167°. It is believed that these preparations were less pure. The resolidified phase exhibited the X-ray powder diffraction diagram recorded by Werner[5] for α-D-galactopyranose. It is probable that an equilibrium between the two anomers was established in the melt. In this equilibrium melt the α-D-pyranose anomer was capable of crystallizing, and as it crystallized the equilibrium in the molten part generated more of it, so that the whole melt solidified as α-D-galactopyranose, which then melted at the higher temperature of 164–167°, slightly below that of the pure α-D-form, 168–170°.

Equal parts of α-D-galactopyranose, m.p. 168–170°, and α-L-galactopyranose, m.p. 168–170°, were crystallized by dissolving in water, concentrating to a sirup and dissolving the sirup in methanol. The resultant optically inactive, crystalline phase melted at 145–148°. Previously reported values are: 163°,[7] 140–142°,[8] 143–144°.[9] The X-ray powder diffraction diagram of this preparation was identical with that of α-D-galactopyranose. This racemic phase is therefore a mechanical mixture of the α-D and α-L anomers.

(7) E. O. von Lippmann, *Ber.*, **55**, 3038 (1922).
(8) E. Fischer and J. Hertz, *ibid.*, **25**, 1247 (1892).
(9) C. Neuberg and J. Wohlgemuth, *Z. physiol. Chem.*, **36**, 219 (1902).

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COMMUNICATIONS TO THE EDITOR

DISCONTINUITIES IN THE ADSORPTION ISOTHERM OF *n*-HEPTANE ON MOLYBDENUM DISULFIDE

Sir:

In the course of a recent investigation at Walker Laboratory of Rensselaer Polytechnic Institute, certain interesting experimental results were found regarding the appearance of discontinuities in the adsorption isotherm of *n*-heptane on molybdenum disulfide. These results, heretofore referred to in a discussion of observed transitions by Young, Beebe and Bienes,[1] were not included in the original publication on water and benzene adsorption on molybdenum disulfide.[2]

Reproducible discontinuities in the adsorption isotherm of *n*-heptane on molybdenum disulfide at 26.0° were observed at a relative pressure near 0.01 when five to ten minutes was allowed for equilibrium between doses. However, when more time was allowed after the initial and succeeding doses, the discontinuity disappeared, and a smooth isotherm, concave to the pressure axis, resulted. If the sample was reactivated and the shorter time between admission of doses resumed, the discontinuity again appeared. This was observed when the sample was previously degassed at room temperature as well as when "activated" at 200°. At higher pressures (above $p/p_0 = 0.05$) the time after each dose could be shortened considerably without effect.

The low pressure portion of an *n*-heptane adsorption isotherm showing the discontinuity as well as the low pressure portion of a smooth isotherm, in which several hours elapsed between the initial and second dose, is shown in Fig. 1. The adsorbent is "activated adsorbent 1" of molybdenum disulfide, as previously described.[2] The desorption isotherms showed the same appearance of discontinuities, except that several small steps were observed in the same pressure range. Numerous experiments with benzene and water adsorption on the same surface showed no vertical rises in the adsorption or desorption curves, but in experiments with toluene adsorbate a vertical rise at $p/p_0 = 0.003$ was obtained when allowing five to ten minutes between doses. The apparatus used was similar to that described by Harkins and Jura.[3]

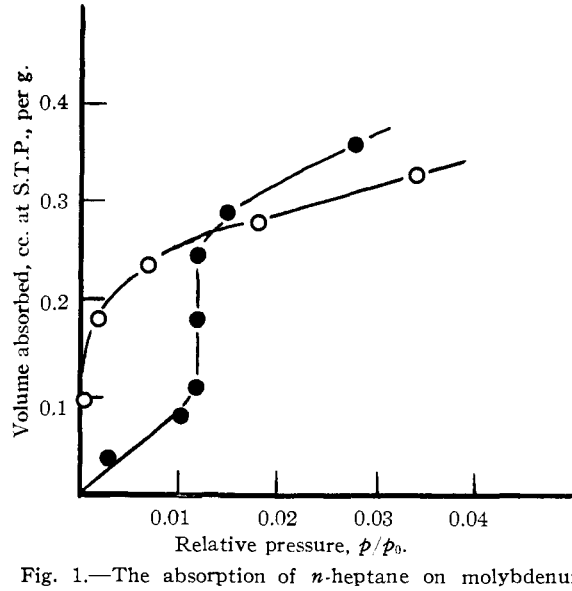

Fig. 1.—The absorption of *n*-heptane on molybdenum disulfide activated adsorbent 1: ●, five to ten minutes between doses; ○, up to eight hours between doses.

Although the appearance of a discontinuity in an isotherm may be a non-equilibrium condition which disappears in sufficient time, this does not eliminate the reality of the observation. As physical adsorption is a rapid process, an adsorbate which

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(2) E. V. Ballou and S. Ross, *J. Phys. Chem.*, **57**, 653 (1953).
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