

physectomized) controls; 1 was a dark (normal) control. In addition many normal controls of other series were available for comparison.

Histologically untreated animals show ovaries with large ovocytes in the central part of the gland but with much interstitial tissue and a cortex which appears to be relatively quiescent in that it consists of many ovogonia and only small numbers of ovocytes. The ovaries of treated animals, on the other hand, are distended with many large ovocytes located in the cortex as well as centrally. The interstitial tissue and the residual nests of ovogonia in the cortex are reduced to relative insignificance. The whole histological picture is that of recent enlargement of numerous ovocytes in the cortex of the gland.

The adrenal seems to be somewhat restored by administration of cortical extract in that the cortical cells seem larger, and definitely to contain more lipid material as shown by treatment with osmic acid. The medullary cells in the treated animals constitute a smaller proportion of the gland. Whether this is relative only, due to enlargement of the cortical cells or to actual reduction of the size of the medullary cells has not been determined.

There is no effect on pigmentation, growth, differentiation or upon the thyroid gland. The effect on the male gonad has not been studied.

6005

The Examination of Urine for Indol.

STUART L. VAUGHAN. (Introduced by R. S. Hubbard.)

From the Buffalo General Hospital, Buffalo, N. Y.

Recently it has been suggested¹ that failure of the liver to conjugate indol may lead to an excretion of that substance in the urine. It was therefore decided to investigate the probable value of the demonstration of indol in the urine as a method of determining the relative efficiency of liver function. In addition to a series of patients with clinical evidence of liver disease others showing marked signs of intestinal putrefaction were studied because it was felt that the large amounts of indol formed in such conditions might overtax the detoxifying power of the liver and so lead to the excretion of the unaltered compound in the urine. Simultaneously

¹ Kendall, A. I., *Arch. Path.*, 1931, **12**, 95.

the question of the interference of indol with the usual test for urobilinogen was investigated; for if appreciable amounts of indol are excreted in the urine, positive tests with Ehrlich's reagent would be obtained which would be indistinguishable from the reaction given by urobilinogen.

In this investigation a sensitive qualitative method based upon the reaction between Ehrlich's benzaldehyde reagent and indol and upon the solubility of the latter compound in petroleum ether was used. Urine was treated with barium chloride to remove bilirubin, filtered and the filtrate tested with Ehrlich's reagent according to the technique of Wallace and Diamond.² If a positive reaction was found which might theoretically be due to indol, to urobilinogen, or to various other compounds, 3 cc. of petroleum ether were added to 15 cc. of the filtrate and the tube inverted several times. The ether layer was then removed with a pipette and tested with Ehrlich's reagent for the presence of indol. Preliminary investigations of the degree of sensitiveness of this method showed that if crystalline indol were added to water or to urine a positive reaction was obtained both in the original solution and in the ether extract made as described when the concentration was one part in 800,000 or more. It was also shown that indol could be completely removed by repeated extraction with petroleum ether when the concentration was much higher than this.

The urine of 3 groups of patients was examined by this technique. The first consisted of 100 hospital patients selected at random. All manner of disease entities were represented. The second consisted of 25 persons whose urine contained large quantities of indican, presumably as a result of marked intestinal putrefaction. In the third group, consisting of 22 patients, a disturbance of liver function was thought to be present. Nineteen cases were jaundiced and showed bilirubin in the urine. Diagnoses made clinically, at operation or by post mortem examination, included acute arsenical hepatitis, acute catarrhal jaundice, subacute yellow atrophy, obstructive jaundice and hemolytic jaundice. In 2 cases jaundice was of undetermined origin. In most of the cases the urine was examined repeatedly during the course of the illness. In no specimen of urine examined was a positive reaction obtained in the ether extract. In no instance, therefore, was indol present in a concentration greater than 1 part in 800,000.

In addition to the test for the presence of indol the urine, after it had been extracted with ether, was in every instance tested with

² Wallace, G. B., and Diamond, J. S., *Arch. Int. Med.*, 1925, **85**, 698.

Ehrlich's reagent according to the technique of Wallace and Diamond. Urine samples which gave a positive reaction before extraction gave the same test after petroleum ether had been used. Petroleum ether, therefore, would apparently serve as a satisfactory method for separating indol from urobilinogen if the former compound occurred in urine. The studies show, however, that need for such a separation occurs seldom, if ever.

In this series containing a fairly large number of patients with intestinal stasis and with jaundice due to various causes, indol could not be demonstrated by a very sensitive qualitative test. While a study of a larger number of extreme conditions might yield some positive results, it is hardly possible that the excretion of indol can serve as a satisfactory method of demonstrating impairment of liver function.

6006

Composition of Bone. XIII. Direct Gravimetric Determination
of Ca, Mg, and PO_4 .

MARTHA L. WASHBURN AND M. J. SHEAR.

From the Pediatric Research Laboratory, the Jewish Hospital of Brooklyn, N. Y.

The literature contains few analyses of bone accurate enough to serve for the determination of the molecular constitution of the calcium phosphate present. The deviations from the "theoretical" composition obtained by previous investigators have usually been ascribed either to shortcomings of the analytical methods employed or to experimental error. Shear and Kramer¹ pointed out the possibility that these discrepancies may have been due to actual variations in the composition of bone; this possibility has not as yet been ruled out.

It is therefore desirable to obtain data by methods capable of differentiating between these possibilities. The standard macro analytical procedures are not suitable for this purpose.

An extended study was made of the determination of Ca, Mg, and PO_4 . Each constituent was studied both in pure solutions and in mixtures. It was found that calcium was completely precipitated in solutions just yellow to thymol blue (pH 3), and that it was completely separated from Mg at this pH. Although both PO_4 and Mg

¹ Shear, M. J., and Kramer, B., *J. Biol. Chem.*, 1928, **79**, 125.