making it impossible to wash out the last traces of hemoglobin. The $QO_2$ values for these preparations are given in Table 1.

TABLE I

| | Nuclei prepared using saponin | Nuclei prepared by Laskowski's method | Nuclei prepared by C citric acid method |
|---|---|---|---|
| $QO_2$ in 0.9% NaCl buffered to pH 7.4 with phosphate, 37.5° C. | 0.40 | 0.10 ±* | 0.10 ± |
| $QO_2$ in 0.11 molar phosphate, pH 6.8, 25° C. | 0.26 | ... | ... |

* Laskowski reports an average value of about 0.20 for the $QO_2$ of his nuclei, with a variation of 0.15 to 0.22. We have made only one determination in duplicate on Laskowski nuclei and our sample may have stood a trifle too long in the ice box before testing.

Nuclei prepared by our method were rather intensely yellow from a pigment which we believe to be largely xanthophyll. To study this pigment the nuclei were washed once with 95 per cent. alcohol, and this washing was discarded. Then the nuclei were extracted three times with 95 per cent. alcohol and the extracts, which contained nearly all the pigment, were combined. The alcohol was evaporated to dryness, leaving a residue of the pigment together with some lipid. This residue was taken up in a small volume of hot alcohol, and on cooling some of the lipid precipitated and was removed. The concentration of alcohol then was lowered to about 92 to 93 per cent. by the addition of water and the pigment was removed from the alcohol by repeated extractions with petroleum ether. These petroleum ether extracts were combined. Subsequent extraction of the petroleum ether with a small volume of 92 per cent. methanol caused the pigment to pass almost quantitatively from the petroleum ether into the methanol layer. The methanol was evaporated to dryness and the pigment, which still contained some lipid, was taken up in carbon disulfide. The carbon disulfide solution gave absorption bands at 444.5, 476.0 and 507.5 mμ, which coincide almost exactly with the positions of bands reported in the literature for xanthophyll.[5] The pigment gave two observable bands in petroleum ether at 444.0 and 475.5 mμ, and in 92 per cent. methanol two bands at 448.0 and 478.0 mμ could be seen. A sample of the pigment that had been evaporated to dryness gave a strong blue color with the Carr-Price reagent.

Although the original alcoholic extract containing the yellow pigment showed practically no fluorescence, a sample of nuclei in which the xanthophyll had gradually disappeared during storage yielded, after drying, a light yellow ether-alcohol extract which was appreciably fluorescent, possibly indicating a flavin pigment.

When the isolated nuclei were suspended in distilled water and the pH was adjusted to about 8.5 with ammonia, a transparent apparently structureless gel was formed which persisted in extreme dilutions. The transparency of this gel is of interest in view of the considerable amount of lipid present. The gel is coagulated to form a rather fibrous precipitate by the addition of a little dilute acetic acid. A similar gel is also formed if 5 or 10 per cent. sodium chloride is added to the centrifuged nuclei, and Laskowski states that saturated sodium chloride solution also causes gel formation. It appears likely that the gel must represent some sort of heavily hydrated lipo-nucleoprotein complex with the lipid in a fairly high degree of dispersion.

SUMMARY

(1) A method has been described for the isolation of chicken erythrocyte nuclei using saponin to lake the cells. Nuclei prepared by this method have been compared with nuclei prepared by the method of Laskowski[2] and with nuclei prepared by a citric acid method, particularly in regard to respiration.

(2) The total lipid content of the nuclei is about 14 per cent. and the desoxyribonucleic acid content is about 45 per cent.

(3) A yellow pigment contained in the nuclei appears to be xanthophyll.

(4) A gel formed by the nuclei at pH 8.5 in the presence of ammonia and the absence of other cations is described.

This work was supported by the International Cancer Research Foundation, of Philadelphia, Pa.

ALEXANDER L. DOUNCE
TIEN HO LAN

## FRACTIONAL CEPHALIN-CHOLESTEROL FLOCCULATION IN HEPATIC DISEASE[1]

SINCE 1938, when Hanger[2] first described the ability of sera from patients with intrahepatic disease to flocculate an emulsion of cephalin and cholesterol, a number of reports have appeared in the literature (summarized in a recent contribution from this institution[3]) indicating the validity of this laboratory procedure as an aid in the study of disorders of the liver. This test has proved to be of particular value in the differentiation between hepatogenous jaundice and

[1] From the Medical Research Laboratory and the Division of Pathological Chemistry, New York Post-Graduate Medical School and Hospital, Columbia University, New York.

[2] F. M. Hanger, *Trans. Asn. Am. Physicians,* 53: 148, 1938; *Jour. Clin. Invest.,* 18: 261, 1939.

[3] C. H. Greene and M. Bruger, *N. Y. State Jour. Med.,* 43: 318, 1943.

[5] H. Gilman, "Organic Chemistry, An Advanced Treatise," Vol. II, page 1149. New York: John Wiley and Sons, 1938.

early obstructive jaundice (*i.e.*, before damage to the hepatic cells has occurred).

The cephalin-cholesterol flocculation test as originally described, however, fails to provide adequate margin for the study of alterations in the degree of damage to the hepatic cells. The purpose of this preliminary report is to show that the degree of cephalin-cholesterol flocculation produced by the addition of various concentrations of serum, when carried out at intervals, may be used as an index of increasing or decreasing hepato-cellular pathology.

The cephalin-cholesterol emulsion[4] was prepared according to the procedure originally described by Hanger.[2] Five chemically clean test-tubes (10 ml capacity) were set up each containing 4 ml of 0.9 per cent. NaCl and 1 ml of the diluted lipid emulsion. To Tube I was added 0.1 ml of the patient's serum and the contents mixed. Decreasing concentrations of serum were then prepared according to the following schedule:

Dilution 1.—0.1 ml of serum diluted with 0.9 ml of 0.9% NaCl.

Dilution 2.—0.1 ml of Dilution 1 diluted with 0.9 ml of 0.9% NaCl.

Dilution 3.—0.1 ml of Dilution 2 diluted with 0.9 ml of 0.9% NaCl.

Dilution 4.—0.1 ml of Dilution 3 diluted with 0.9 ml of 0.9% NaCl.

To Tubes II, III, IV and V were added 0.1 ml of Dilutions 1, 2, 3 and 4, respectively. The tubes were placed in a rack and kept at room temperature for 24 hours. The degree of flocculation of the cephalin-cholesterol emulsion was then read and graded from 0 to ++++, the former indicating no flocculation and the latter complete clearing of the supernatant fluid.

The table shows the results obtained in two of seven patients with simple (catarrhal) jaundice. In Case I. F., fractional cephalin-cholesterol flocculation tests carried out at intervals indicated increasing hepatic damage from the day of admission to the hospital until the 34th day when the icterus index reached 188 units. Clinical improvement was evident on the 48th hospital day when flocculation failed to occur in Tubes IV and V. In this patient, the extent of fractional flocculation appeared to vary directly with the icterus index. In Case D. C., clinical improvement was accompanied by decreased to absent flocculation in the high dilutions; of interest was the lag observed in the diminishing cephalin-cholesterol flocculation compared to the decided decrease in the icterus index on the 28th hospital day. This is not unlike the lag in the return of the erythrocyte sedimentation rate to normal commonly encountered in patients recovering from acute rheumatic fever.

[4] In these studies, the cephalin-cholesterol antigen prepared by the Difco Laboratories, Detroit, Michigan, was used.

TABLE I

FRACTIONAL CEPHALIN-CHOLESTEROL FLOCCULATION IN TWO PATIENTS WITH SIMPLE (CATARRHAL) JAUNDICE

| Days | Cephalin-Cholesterol Flocculation | | | | | Icterus Index |
|---|---|---|---|---|---|---|
| | Tube I (0.1 ml serum) | Tube II (0.01 ml serum) | Tube III (0.001 ml serum) | Tube IV (0.0001 ml serum) | Tube V (0.00001 ml serum) | |
| | Case I. F. Female, age 45 years | | | | | |
| 0 | ++++ | ++++ | +++ | 0 | 0 | 94 |
| 11 | ++++ | ++++ | +++ | 0 | 0 | 125 |
| 17 | ++++ | ++++ | +++ | ++ | 0 | 150 |
| 34 | ++++ | ++++ | +++ | + | + | 188 |
| 42 | +++ | +++ | ++ | + | + | 150 |
| 48 | +++ | +++ | + | 0 | 0 | 107 |
| | Case D. C. Female, age 46 years | | | | | |
| 0 | ++++ | +++ | +++ | ++ | 0 | 125 |
| 28 | ++++ | +++ | +++ | + | 0 | 21 |
| 49 | +++ | +++ | ++ | 0 | 0 | 13 |

*Summary:* The fractional cephalin-cholesterol flocculation test appears to be a valid procedure for following alterations in the degree of hepato-cellular damage in patients with diseases of the liver. When carried out at intervals, the fractional test permits the evaluation of injury to the hepatic cells in terms of increasing or decreasing pathology.

MAURICE BRUGER
NEW YORK POST-GRADUATE MEDICAL SCHOOL AND HOSPITAL

## STUDIES ON PERIPHERAL VISUAL ACUITY

ALTHOUGH peripheral visual acuity curves are well known, there has been very little work done to determine normal variability or to correlate this acuity with other visual functions. Certain wartime accidents under combat conditions have indicated that faulty peripheral acuity, unknown to the individual, may have been responsible. This prompted the writer to develop a reasonably short, accurate test of this visual function and to investigate the possibility of improving it by systematic training. A brief report is presented herewith.

Peripheral visual acuity (or efficiency) was measured by the use of Landolt broken circles with breaks of the following sizes; .5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9 and 10 mm, fitted to a carrier mounted on a 25 cm perimeter. Illumination was kept constant with a built-on illuminator, the light from a 60-watt Mazda daylight lamp 35 cm from the test object being incident at 45°. Surrounds and operator were blacked out. Nine points on each eye, 30°, 60° and 90° from the line of vision, were tested with the other eye blacked out. The test object was covered with a black paddle and then revealed successively in any of four possible positions corresponding to points of the compass (N, S, E or W), the subject signaling the position with the eye fixed forward. Four consecutive successful identifications were necessary for a score. The