Ordinarily, necrosis refers to changes in cells after death while in the body(13) but autolysis is usually used to describe specific intracellular effects of enzyme action on cells either in or removed from the body. These terms were used somewhat synonymously by Berenbom *et al.*(3,4) in recent studies. These studies dealt with chemical changes associated with mouse liver necrosis *in vivo* and *in vitro*. Ribonucleic acid (RNA) showed the most dramatic change, being reduced to 4% of its initial value 48 hours after pieces of liver were placed in the peritoneal cavity. DNA and protein nitrogen decreased much more gradually than did RNA. However, rate of disappearance of DNA and protein nitrogen was greater *in vitro* than *in vivo*. The fact that initial autolytic changes in kidney and adrenal gland in our study produce larger nuclei and are followed by decrease in nuclear size suggests that more than one cell constituent is undergoing change.

Summary. Small pieces of mouse kidney, liver, and whole adrenal glands were allowed to autolyze in incubator for varying times before fixation. Nuclear sizes were measured and compared with control tissues that had been mounted and stained together on the same slide. Nuclear volumes increased about 70% over controls during the first 30 to 60 minutes of autolysis in kidney and adrenal cortex. This was followed by a decrease in volumes eventually leveling off to about half that of the control in the kidney and the same as the control in adrenal cortex. A comparison of frequency distribution of nuclear volumes in liver revealed no differences between autolyzed samples and the control samples.

The author acknowledges the assistance of Robert L. Smith, Jr., who worked out the statistical analyses on the IBM 650.

1. Jacoby, M., *Z. f. physiol. Chem.,* 1900, v30, 149.
2. Leuchtenberger, C., *Chromosoma,* 1950, v3, 31.
3. Berenbom, M., Chang, P. I., Betz, H. E., Stowell, R. E., *Cancer Res.,* 1955, v15, 1.
4. Berenbom, M., Chang, P. I., Stowell, R. E., *Lab. Invest.,* 1955, v4, 315.
5. Kasten, F. H., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 493.
6. ———, *J. Nat. Cancer Inst.,* 1955, v16, 579.
7. Stowell, R. E., *Stain Tech.,* 1945, v20, 45.
8. Salkowski, E., *Z. f. klin. Med.,* 1890, Suppl. to v17, 77.
9. Wells, H. G., *Chemical Pathology,* 4th Ed., 1920, Saunders, Philadelphia.
10. Karsner, H. T., *Human Pathology,* 6th Ed., 1942, Lippincott, Philadelphia.
11. Roslansky, and Alfert, quoted by Alfert, M., *Biol. Bull.,* 1955, v109, 1.
12. Alfert, M., *ibid.,* 1955, v109, 1.
13. Boyd, W., *Textbook of Pathology,* 3rd. Ed., 1938, Saunders, Philadelphia.

Received March 24, 1958. P.S.E.B.M., 1958, v98.

Reaction of Brain Copper Proteins with Sodium Diethyldithiocarbamate in Normal and in Hepatolenticular Degeneration.* (24015)

HUNTINGTON PORTER AND SALLY AINSWORTH (Introduced by Byron H. Waksman)
Neurology Service, Mass. General Hospital and Dept. of Neurology, Harvard Medical School, Boston, Mass.

A previous report(1) described the separation from brain tissue under copper-free conditions of 3 different copper-containing fractions: fraction I, extracted from fresh tissue with 0.1 M acetate buffer pH 4.5 (or with water or with 0.1 M bicarbonate buffer pH 8.2); fraction II, obtained by subsequent extraction of tissue residue with water at pH 3.5 and vanishing ionic strength; and the residual fraction III. In brains from 2 cases of hepatolenticular degeneration, each with total copper content increased to more than 13-fold the normal, more than ⅔ of the pathological brain copper was extracted in fraction I in a form quantitatively undialyzable at pH

* This investigation was supported by Research Grant B-921(c) from Nat. Inst. of Neurological Diseases and Blindness, U.S.P.H.S.

8.2, presumably bound to proteins. Cerebrocuprein I(2), an essentially homogenous brain protein containing 0.25 to 0.3% copper, has been isolated from bovine fraction I. The copper in cerebrocuprein I was observed to react directly with sodium diethyldithiocarbamate when this reagent was added directly to a solution of copper-containing protein without previous digestion. Since the copper in both ceruloplasmin(3) and hepatocuprein(4), 2 other copper proteins in which this reaction has been studied, fails to react directly, it was considered possible that the direct reaction exhibited by cerebrocuprein I might have been an artifact due to modification of properties of the naturally occurring brain copper protein by the rather rigorous procedures employed during its isolation. Attempts to investigate this possibility by determining the direct-reacting copper in fresh fraction I extracts of bovine brain were unsatisfactory because of the relatively low copper concentration of such extracts in proportion to the abundance of heme compounds also present, and the high absorption of the latter at the wave length employed in measuring the direct reaction. The present paper describes further study of the extent to which the copper in crude bovine cerebrocuprein I prepared by a less drastic method and in fraction I copper proteins from normal human brain and brains from 2 cases of hepatolenticular degeneration reacts directly with sodium diethyldithiocarbamate.

Methods and material. Precautions to avoid contamination with extraneous copper, the general conditions of fractionation procedures, criteria for selection of normal human brains for fractionation, and the methods used for tissue homogenization and for copper analysis were all identical with those employed in previous work(1,2). The direct-reacting copper was determined by a modification of the procedure of Gubler *et al.*(5). Aliquots of a solution of the brain fraction to be analyzed were placed directly in the spectrophotometer cuvette, sufficient redistilled water was added to make the total volume 3.25 ml, 0.5 ml of a saturated solution of sodium pyrophosphate were added, and the optical density (D_1) at 445 mμ measured in Coleman Junior spectrophotometer. 0.25 ml of a 0.1% aqueous solution of sodium diethyldithiocarbamate were added, the solution thoroughly mixed, and the optical density (D_2) measured at 10-minute intervals for the following 60 minutes. By the end of this period, the optical density had in all cases ceased to change significantly. The change in optical density of a reagent blank (D_3) after addition of carbamate was also measured. The net change in optical density 60 minutes after addition of carbamate (Δ OD) was calculated as $D_2 - (D_1 \times 0.938 + D_3)$ and the corresponding quantity of copper calculated from a standard curve. 89 to 110% of copper added to protein samples before pyrophosphate could be recovered as direct-reacting copper. Crude bovine cerebrocuprein I (Cu content 0.15%) was prepared by the procedure previously described(2) as method A (including preparative paper electrophoresis) with the following modifications: (a) acetone precipitates were not dried; (b) dialysis was carried out for only 24 hours, but with constant mechanical shaking; (c) the precipitation at pH 8.2 and the third and fourth acetone precipitations were omitted; (d) the second acetone precipitation was carried out after addition of 1/10 volume of 0.1 M acetate buffer pH 5.2 to the dialyzed solution. The brains of the 2 cases of hepatolenticular degeneration, described previously(1), had been stored at −15° as frozen whole brain (Case A) or as lyophilized dialyzed bicarbonate extract (Case B). The normal human brains had been stored at −15° for 3 months. Copper proteins in pH 4.5 and pH 5.2 extracts from normal human brain were concentrated by precipitation with 3 volumes of acetone and the precipitates dissolved in sufficient 0.2 M ammonium bicarbonate to make a 5 to 10% protein solution. A fraction containing 0.14% copper(6) was obtained from pH 8.2 extracts of normal human brain by dialysis, addition of 1/10 volume of 0.1 M acetate buffer pH 5.2 and collection of the material precipitating between 50% and 75% (v/v) acetone concentration. The pH 8.2 extracts from the 2 cases of hepatolenticular degeneration were analyzed without previous

TABLE I. Direct Reaction of Copper in Fraction I Brain Copper Proteins with Sodium Diethyldithiocarbamate.

Species and preparation	μg Cu in sample analyzed	Extent of direct reaction as Δ O.D.*	as μg Cu	as % of total Cu in sample
Bovine crude cerebrocuprein I	7.25	.013	.47	7
Idem	4.63	.012	.42	9
Normal human Fraction I				
As pH 4.5 extract	7.60	.027	.97	13
Idem	9.12	.028	1.03	11
As pH 5.2 extract	5.88	.013	.47	8
" " 8.2 " †	20.00	.018	.65	3
Hepatolenticular degeneration Fraction I				
Case A as pH 8.2 extract	4.86	.039	1.45	30
Idem	10.02	.078	2.90	29
Case B as pH 8.2 extract	11.25	.106	3.92	35
Case B repeated‡	20.12	.242	9.00	45

* Net change in optical density 60 min. after addition of sodium diethyldithiocarbamate.
† Material precipitating between 50% and 75% of acetone concentration after dialysis of pH 8.2 extract and addition of 1/10 vol of 0.1 M acetate buffer pH 5.2.
‡ Repeated on extract stored at −10° for 41 days.

acetone precipitation.

Results. The extent of the direct reaction found in crude bovine cerebrocuprein I, in normal human fraction I obtained by extraction of the tissue at various pH's and in fraction I from 2 cases of hepatolenticular degeneration is shown in Table I. Since the sample of cerebrocuprein I analyzed was only about 50% pure, as indicated both by its copper content of only 0.15% and by ultracentrifugation studies,† it seems probable that the observed direct-reacting copper, amounting to from 7 to 9% of total copper in samples, may represent copper other than that attached to cerebrocuprein I and that the copper in pure cerebrocuprein I does not react directly. The present evidence indicates that the previously observed direct reaction of cerebrocuprein I was an artifact due to the rigorous procedures of isolation and that cerebrocuprein I is, in fact, similar to ceruloplasmin and hepatocuprein in having a copper-protein bond of a type which retains copper against the competing action of sodium diethyl-dithiocarbamate. More than 85% (2.1 to 2.4 μg/g fresh tissue) of the copper in normal human fraction I also appears to be bound in a form which renders it incapable of reacting directly. It is uncertain whether the small amount of direct-reacting copper (<0.35 μg/g fresh tissue) found in this fraction is naturally occurring and of physiological significance as has been shown for the small percentage of plasma copper reacting directly(5).

In brains from 2 cases of hepatolenticular degeneration 29 to 45% of fraction I copper was found to react directly. This observation supports evidence previously presented(1) that a significant portion of the increased brain copper in this disease is bound in a form different from that comprising the bulk of normal human fraction I copper. The direct-reacting copper in fraction I from cases of hepatolenticular degeneration (>7.5 μg/g fresh tissue *i.e.* more than 20-fold the amount of direct-reacting copper in normal human fraction I) may represent copper bound to brain proteins which are normally copper-free. A large part (>17 μg/g fresh tissue in the 2 cases examined) of the pathological copper accumulating in the brain in this disease, however, appears, like the major portion of the copper in normal human brain, to be bound in a form which renders it incapable of reacting directly with sodium diethyldithiocarbamate.

† Carried out through courtesy of Prof. J. L. Oncley of Harvard Medical School.

Summary. 1) Less than 15% of copper in crude bovine cerebrocuprein I (Cu content 0.15%) and in fraction I copper proteins from normal human brain reacted directly with sodium diethyldithiocarbamate. 2) In brains from 2 cases of hepatolenticular degeneration 29 to 45% of the fraction I copper reacted directly. This direct-reacting copper, amounting to more than 7.5 μg/g fresh tissue *i.e.* more than 20-fold the amount of direct-reacting copper in normal human fraction I, may represent copper bound to brain proteins which are normally copper-free.

1. Porter, H., Folch, J., *Arch. Neurol. Psychiat.*, 1957, v77, 8.
2. ———, *J. Neurochem.*, 1957, v1, 260.
3. Holmberg, C. G., Laurell, C. B., *Acta Chem. Skand.*, 1948, v2, 550.
4. Mann, T., Keilin, D., *Proc. Roy. Soc. B.*, 1938, v126, 303.
5. Gubler, C. J., Lahey, M. E., Cartwright, G. E., Wintrobe, M. M., *J. Clin. Invest.*, 1953, v32, 405.
6. Porter, H., Folch, J., *Proc. First International Congress of Neurological Sciences*, Pergamon Press, London.

Received March 24, 1958. P.S.E.B.M., 1958, v98.

Effect of Tolbutamide on Serum Cholesterol Levels in Hypercholesterolemic Patients.* (24016)

WILLIAM R. WADDELL (Introduced by F. J. Stare)
Department of Nutrition, Harvard University School of Public Health, Boston, Mass.

On the basis of the mild abnormality in glucose tolerance in most patients with idiopathic hypercholesterolemia, the suggestion has been made that these patients have an abnormality of their energy metabolism that resembles diabetes(1). Because of this, a trial of tolbutamide has been carried out to determine what effect, if any, such treatment would have on the elevated serum cholesterol levels. Eight patients were studied. Control periods of one to 24 months were available, since these patients were selected from a larger group being studied because of hypercholesterolemia. Total serum cholesterols ranged between 300 and 500 mg/100 ml. Tolbutamide administration was begun with 3 g the first day, 2 g the second day and 1 g daily thereafter for 2 months. A diet containing approximately 50 g fat/day was constant throughout control and experimental periods. The patients' weight did not fluctuate more than 2 pounds above or below that at beginning of the experimental period. Total and esterified serum cholesterol determinations were made after one week of treatment and at one or 2-week intervals thereafter.

Results. In 5 of the 8 patients a drop in serum cholesterol levels occurred after one week. In one instance this decrease amounted to 100 mg/100 ml of serum and in the others was approximately 50 mg. In 3 patients there was no change from the control period. After the first week there was a slight rise above control values in 5 patients, and no change in the other 3. All patients that showed a decrease after the first week returned to control value or higher by the second or third week and then cholesterol concentration fluctuated at or above control levels until the end of the 2-month period. In no patient was the serum cholesterol lowered below control levels during the second month of tolbutamide therapy.

Conclusions. Tolbutamide administered to hypercholesterolemic patients in doses of 1 g daily did not result in lowering of serum cholesterol concentrations although temporary reductions occurred in 5 of 8 patients during the first week of drug administration.

* Tolbutamide (Orinase) was supplied by Upjohn Co., Kalamazoo, Mich., through the courtesy of Dr. E. A. Hawk.

1. Waddell, W. R., Geyer, R. P., Hurley, N., Stare, F. J., *Metabolism*, 1958, in press.

Received March 24, 1958. P.S.E.B.M., 1958, v98.