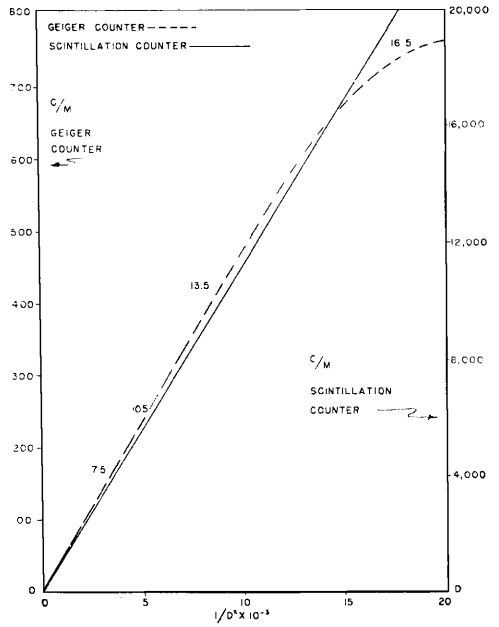

FIG. 3.
Comparison of scintillation and Geiger counter detection at various distances.

A comparison of the two counting rates is shown in Fig. 3 in which the counting rate is plotted versus the inverse square of the distance. At a distance of 7.5 inches, the Geiger counter is seen to deviate from the inverse square law while the scintillation counter is still accurate. The standard of I^{131} was measured in the above case in 50 cc of water to approximate roughly the conditions of actual measurement.‖

Summary. 1. The problems inherent to the measurement of I^{131} uptake in the thyroid gland have been reviewed and the applicability of the scintillation counter for this use discussed. 2. The selection of scintillation phosphor and construction of the counter has been described and compared in operation over a 6-weeks period with a conventional Geiger counter. This improvement of sensitivity has shown to be a factor of 30 ~ 50. 3. The increased sensitivity described has shown to be sufficient to allow an immediate reduction of the tracer dose from 100 μc to 50 μc, with a recent reduction to 25 μc. It may be seen that the dose could be reduced to 10 μc with considerable ease. At the same time a decided improvement over the Geiger counter in counting statistics has been obtained. Based on the previous counting rates the 10 μc standard would exhibit a counting rate ~ 2,700 c/m and an uptake rate ~ 1,200 c/m. Background for such measurements would be 200 ~ 400 c/m.

‖ These clinical measurements were made by John P. Storaasli, M.D. Department of Radiology, University Hospitals, Cleveland, Ohio.

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Effect of Intravenous Injections of Casein Hydrolysate upon Electrocorticogram of the Rabbit.* (18267)

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(Introduced by J. L. O'Leary)

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Enzymatic casein hydrolysate is widely used in the intravenous feeding of protein depleted patients(1). If injected too rapidly side effects of nausea, vomiting and headache ensue. To test the possibility that these symptoms arise from central excitation, a commercial aqueous solution of enzymatic casein hydrolysate† (10% weight/volume,

* Aided by a grant from U. S. Veterans Administration for research in epilepsy.

1. Elman, Robert, Parenteral Alimentation in Surgery with Special Reference to Proteins and Amino Acids. Paul B. Hoeber, New York, 1947.

† The enzymatic casein hydrolysate was provided by Mead, Johnson and Co. We are indebted to Drs. K. S. Kemmerer and W. M. Cox of that company for extensive assistance in preparation of the concentrate.

nitrogen content 1.2%) was given intravenously to rabbits while the electrocorticogram was being recorded. The solution was given at a standard rate of 0.3 to 0.5 ml per second and the minimum amount necessary to produce a change in brain activity was determined. Thereafter the amino acids and peptides in the solution were fractionated by adsorption on Amberlite I-R-4, an acid binding resin(2). Both eluates and effluents were tested in rabbits. Finally, samples of the initial solution and its most active fraction were compared by 2-dimensional paper partition chromatography(3).

The fraction which showed the most marked central effect, as judged by change in brain activity, was obtained as follows: to 1 liter (100 g dry weight) of the enzymatic hydrolysate hydrochloric acid was added to bring the pH to 4.5. The solution was then thoroughly stirred into the 40 g of Amberlite I-R-4 in an evaporating dish and the mixture allowed to stand for 1 hour. The resin was filtered off, washed and eluted with N sodium hydroxide until a pH of 7 was reached; this eluate showed no significant central effect. The resin was eluted again at pH 11.5. The eluate was neutralized with HCl to pH 7 and concentrated to a nitrogen content of approximately 1%. Three separate batches of this maximally active fraction were prepared and a similar effect on cortical activity was produced by each.

Fifty-seven adult rabbits lightly anesthetized with ether were used in the tests. In each animal the cerebral hemispheres were widely exposed for transcortical recording with an ink-writing electroencephalograph. After a 30-minute control electrocorticogram, the test solution was injected at a uniform rate into an ear vein until a change in pattern was obtained, as denoted by the appearance of high voltage paroxysmal discharge, or by assumption of isopotential character (*i.e.*, flattening). In some animals convulsions accompanied the paroxysmal discharge, and death might occur either with or without preceding convulsive activity.

Findings. When the source solution of enzymatic casein hydrolysate was used, changes in the electrocorticogram occurred in 19 of 24 trials with an average injection of 44.3 ml. By contrast the most active fraction, given to 10 animals in 16 trials, required an average injection of only 9.3 ml to produce an effect. Injection of this fraction was followed in 15 trials by marked changes in the recorded brain wave activity in 8 instances with convulsions, and in 4 by death. Effluents were uniformly ineffective, and eluates obtained at other pH's were significantly less active.

Various control solutions were essentially without effect on the electrocorticogram under the standard test conditions. These included: isotonic sodium chloride; unbuffered acid (HCl) and alkaline (NaOH) solutions of pH 3, 5, 7, and 10; 5 and 10% solutions of glucose in water and in physiological saline; solutions of glutathione, glutamic acid, amino acid mixture (10%), acid hydrolyzed fibrin, and acid hydrolyzed casein hydrolysate.

The composition of the enzymatic casein hydrolysate and of the most active fraction (the eluate at pH 11.4) were studied by two dimensional paper-partition chromatography before and after acid hydrolysis.‡ The amino acids were identified by comparison with a reference chromatogram of Dent(3). After the acid hydrolysis of the enzymatic hydrolysate, spots corresponding to glutamine and to a ninhydrin-reactive unknown located below aspartic acid disappeared. On the other hand, the glutamic acid, aspartic acid and valine spots became darker and cystine (cysteic acid), methionine (methionine sulfone) and tyrosine appeared, presumably because these were liberated from peptides which had escaped enzymatic hydrolysis.

The pattern of amino acids found in the pH 11.4 eluate was markedly different from that of the source solution. The quantities of glutamic and aspartic acids were larger and there were several unidentified spots below aspartic acid which gave a strongly positive ninhydrin reaction.

2. Cannan, R. K., *J. Biol. Chem.*, 1944, v152, 401.
3. Dent, C. E., *Biochem. J.*, 1948, v43, 169.

‡ We are indebted to Dr. E. Roberts of the Department of Anatomy for preparing and interpreting the chromatograms.

The unidentified spots all disappeared following the acid hydrolysis of this fraction. The chromatogram then showed further increases in the concentrations of glutamic and aspartic acids, but other amino acids were also increased. There was no evidence that basic amino acids were present. Their absence is consistent with the fact that Amberlite I-R-4 is an acid-binding resin.

Conclusions. An enzymatic casein hydrolysate was found to contain an agent which produced significant changes in the electrical activity of the rabbit brain when administered under standard test conditions. The agent appeared to be a protein fragment which is adsorbed by Amberlite I-R-4 and yields large amounts of glutamic and aspartic acids when destroyed by acid hydrolysis.

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Influence of Age on Hepatic Response to Choline and Cystine Deficiency in the Rat.* (18268)

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During the past 15 years there has accumulated a considerable body of literature describing the hepatic sequelae of choline and cystine deficiencies. Unfortunately, this literature has been complicated by the fact that the many investigators in this field have employed rats of different strains and ages as well as diets of quite different composition. The present paper describes an attempt to determine the extent to which the initial age of the rat modifies the hepatic response to choline and cystine deficiency in one strain of rats fed one variety of deficient diet.

Experimental. The rats used in these studies were males of the Vanderbilt strain (1). All animals were grown to the desired weight on a commercial feed, housed in individual cages and offered the experimental rations and water *ad libitum*. The basal diet consisted of acid and alcohol washed casein 5, corn starch 54, cottonseed oil 10, cod liver oil 4, shortening 20, salts(4) 4, cholesterol 0.5, cystine 0.6 and choline chloride 0.8. Deficient diets were prepared by simply omitting one of these constituents. In addition, each diet contained the following in mg/kilo of diet mixture: Thiamine 5, riboflavin 8, pyridoxine 5, calcium pantothenate 30, *p*-aminobenzoic acid 75, α-tocopherol 25, biotin 1, pteroylglutamic acid 2, naphthohydroquinone acetate 5, niacin 50. These diets were chosen because of the considerable body of previous experience(2,3) with choline and cystine deficiency in rats fed such rations. With each deficient diet, rats of 3 ages were employed whose initial weights were 50, 150 and 300 g. Since previous experience had indicated that older rats were comparatively resistant to these deficiencies, the experiment was planned in such fashion that, for each of the 3 diets, a representative group of the older rats was sacrificed at about the time the youngest rats on the diet had died. The remaining rats were maintained on the diets and, finally, those which survived were sacrificed after they had been on the diet for 270 days. All livers were fixed in formalin, sectioned and stained with hematoxylin and eosin. On ex-

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2. Handler, P., and Follis, R. H., Jr., *J. Nutrition,* 1948, v35, 669.

3. Handler, P., and Dubin, I. N., *J. Nutrition,* 1946, v31, 141.

4. Hubbell, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutrition,* 1937, v14, 273.