Sera from cases of encephalitis in Japan have now been tested for a similar effect. Professor Inada, of Tokyo, kindly sent us sera from 3 persons with a history of encephalitis in August, 1924, aged at that time 60, 50 and 51 years, respectively, and from 9 persons with a similar history in August and September, 1933, aged 17, 17, 20, 26, 33, 46, 53, 62 and 65 years, respectively. In each case fever was noted for 6 to 9 days. The sera were drawn January 10 to 12, 1934. Professor Takaki, of Tokyo, likewise sent us sera from 3 cases from the August, 1933, outbreak. None of these 15 sera showed any protective action against the virus of the St. Louis disease.

Experiments have also been made on the ability of the encephalitis virus to incite a specific immunity in the mouse. Active brain virus given to mice intranasally in doses as small as 10^{-5} gms or intracerebrally in 10^{-8} gms causes death, while the same virus-containing material injected intraperitoneally or subcutaneously in 10^{-2} gm amounts in $\frac{1}{2}$ cc of diluent (which is a million intracerebral and a thousand intranasal lethal doses) rarely proves fatal. Still smaller amounts, 0.001, 0.0001, 0.00001 and 0.000001 gm, when inoculated subcutaneously, induce no symptoms but render them immune to a million intracerebral and a thousand intranasal doses. This induced active immunity has persisted unchanged for 3 weeks and doubtless endures much longer.

This report, therefore, indicates first, that the Japanese B. type and the St. Louis form of epidemic encephalitis are serologically distinguishable; and second, that animals as highly susceptible to infection as are mice, by certain portals of entry of the virus, may be immunized actively by the introduction of minimal amounts of virus—into parts of the body more refractory to its pathogenic action.

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AN ATTEMPT TO ISOLATE VITAMIN A

UP to this time the most powerful concentrate of Vitamin A ever known was a pale yellow oil prepared from a liver oil by P. Karrer and his associates at Zurich. As he measured its potency with the antimony trichloride color reagent it was approximately 10,500 times as rich in Vitamin A as the standard cod-liver oil.

Karrer and Morf,[1] in a recent paper on this subject, refer to a rich concentrate prepared by Carr and Jewell[2] with a very high cod-liver oil value ("C. L. O. value") of 7,800 and to another preparation by Heilbron, Heslop, Morton, Webster, Rea and Drummond[3] with a C. L. O. value of 6,500. These English workers used high-vacuum distillation methods.

In the Severance Laboratory at Oberlin College we have recently obtained a much richer concentrate of Vitamin A than those listed above. Repeated experiments have yielded products with C. L. O. values ranging from 13,000 to approximately 14,000. A convincing number of products ranked above the 10,500 value previously supposed by many to represent pure Vitamin A. Until we can crystallize our richest product, however, we would not be justified in calling it the isolated vitamin, yet it seems probable that it is extremely close to 100 per cent. pure.

The Parke Davis Company and the Abbott Company generously supplied us with the non-saponifiable portion of halibut liver oil as a starting material. This was chilled in methyl alcohol solution, to freeze out cholesterol, etc., filtered cold under nitrogen, transferred to pentane by addition of water, dried over anhydrous sodium sulfate, and then in pentane solution cooled to about $-70°$ C. with the aid of carbon dioxide snow mixed with alcohol and again filtered with careful exclusion of oxygen to remove impurities frozen out. This procedure was, in general, that of Karrer, but from this point on variations were introduced.

The cold pentane solution was next filtered through a Tswett column of very specially prepared carbon (Karrer used alumina and lime), and washed completely through with pure pentane.

The method of Karrer involved washing the strongest color band into the middle section of his column,

TABLE I

VITAMIN A CONCENTRATES RANGING FROM 14,400 TO 10,500 TIMES THE POTENCY OF STANDARD COD LIVER OIL

No.	C.L.O. value	Worker	No.	C.L.O. value	Worker
1.	14,400	Manly	14.	11,400	Hartzler
2.	13,500	"	15.	11,300	"
3.	13,500	Hartzler	16.	11,300	"
4.	12,900	"	17.	11,300	Manly
5.	12,600	"	18.	10,800	Hartzler
6.	12,500	"	19.	10,700	"
7.	12,000	Manly	20.	10,600	"
8.	12,000	"	21.	10,600	"
9.	12,000	"	22.	10,600	Manly
10.	12,000	Hartzler	23.	10,600	"
11.	11,800	"	24.	10,500	Hartzler
12.	11,800	Manly	25.	10,500	"
13.	11,600	"			

NOTE:
Tswett Column I. includes filtrate fractions 1, 2, 7, 13.
" " II. " " " 3, 4, 5, 6, 14, 15, 21, 24, 25.
" " III. " " " 8, 9, 12, 17, 22, 23.
" " IV. " " " 10, 11, 16, 18, 19, 20.

[1] *Helv. Chim. Acta*, 16: 625, 1933.
[2] *Nature*, 131: 92, 1933.
[3] *Biochem. Jour.*, 26: 1178, 1932.

followed by the cutting out of this section and the extraction of the vitamin from this rich portion.

Our pentane fractions were analyzed by the "Official Pharmacopoeia Method" reported by the Cod Liver Oil Color Sub-Committee, using the usual Lovibond tintometer. One worker had used such methods for a year and the other was approved after frequent comparisons.

Needless to state, there are many precautions and many difficulties in working with a substance so extraordinarily sensitive to oxidation (and to light), but these will be discussed in a later paper. It was thought best to make this preliminary announcement before taking time to analyze the concentrate, to determine its molecular weight, its spectral absorption bands, its extinction coefficients and its biological value.

Like the other workers mentioned, we secured a pale yellow, viscous oil. We shall continue our attempts to crystallize it.

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THE EFFECT OF X-RAYS ON GROWTH SUBSTANCE AND PLANT GROWTH

IT has long been known that the growth of plant and animal tissues is inhibited by x-radiation. The amount of inhibition has generally been found to be a function of the amount of irradiation up to a certain lethal dosage which varies greatly with different types of cells and under different experimental conditions.

In this preliminary report of work done during the last year on the effect of x-rays on growth substance, the "growth hormone" of plants, it is possible to explain the immediate effects of irradiation in terms of its action on a specific chemical substance. The high voltage x-ray tube of the W. K. Kellogg Radiation Laboratories of the California Institute of Technology, which produces a well-defined beam of hard γ-rays of uniform intensity (soft rays being eliminated by successive steel, lead and aluminum filters) was used. The dosages given varied from 20 to 5,000 Röntgen units applied at different rates between 15 and 50 Röntgen units per minute at 750,000 to 925,000 volts and 3 to 4 milliamperes. The growth substance was irradiated in different media and solvents, such as agar blocks of standard size and concentration, water and other solutions of known activity. A few species of plants were directly irradiated. The amount of growth substance remaining after irradiation was determined by the usual *Avena* technique.

Growth substance was inactivated by irradiation both in solution and in the intact plant and by comparable dosages. Under certain conditions the inactivation was complete. When a water solution of growth substance was irradiated in an atmosphere of nitrogen there was no immediate inactivation, indicating that the reaction is an oxidation.

Tips from irradiated plants, placed on agar blocks, showed a large decrease in growth substance diffusing out from them as compared with tips from control plants. This was true even when relatively small dosages were administered, so that the growth rate of the intact plant would be only temporarily decreased. This difference in amount of growth substance diffusing out from the tip is not due to an effect produced by irradiation on the rate of growth substance transport through the plant, because the transport is not decreased by previous irradiation of the plant.

Irradiated seedlings showed a decrease in growth rate. But similar seedlings, given a continuous supply of growth substance immediately after irradiation, were able to maintain their normal growth rate compared with similarly treated nonirradiated controls. No stimulation of growth by irradiation was observed for the intensities used in these experiments.

Many workers have found that regeneration in plants through the development of buds and shoots is greatly increased by moderate dosages of x-rays. Since it is known that growth substance inhibits the outgrowth of buds, this regeneration may now be explained by the inactivation of growth substance by x-rays, rather than interpreted as a stimulative effect on the cells irradiated.

These experiments show that the primary action of x-rays on the growth of plants is through the inactivation of growth substance. They offer a simple technique whereby the immediate effects of irradiation on the living cell may be quantitatively studied.

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BOOKS RECEIVED

DOWNING, ELLIOT R. *An Introduction to the Teaching of Science.* Pp. vii + 258. University of Chicago Press. $2.00.

MEYERHOFF, HOWARD A. *Geology of Puerto Rico.* Pp. 306. 45 figures. The University of Puerto Rico.

PACKARD, EARL L., REMINGTON KELLOGG and ERNST HUBER. *Contributions to Palaeontology: Marine Mammals.* Pp. 136. Illustrated. Carnegie Institution of Washington.

Report of the Secretary of the State Board of Agriculture and of the Experiment Station of the State of Michigan, 1931–1932. Pp. 288. 29 figures. Board of Agriculture of the State of Michigan.

SIERPINSKI, WACLAW. *Introduction to General Topology.* Translated by C. Cecilia Krieger. Pp. x + 235. University of Chicago Press. $4.00.

TIMOSHENKO, S. *Theory of Elasticity.* Pp. xvi + 416. 203 figures. McGraw-Hill. $5.00.