trypsin, pepsin, and various peptidases are described in detail almost as great as that found in Northrup's book on Crystalline Enzymes, but the methods for the crystallization of papain and ficin are not included.

The discussion of each enzyme begins with a short historical note followed by a list of organs or organisms where the enzyme has been found. With the exception of urease no data are given to indicate the relative concentration of the enzyme in the cells where it occurs. The action and specificity are then discussed and excellent formulae and equations are presented. This is usually followed by a description of the preparation and purification of the enzyme and a very useful outline of the methods for the estimation of its activity. The compounds which inhibit the enzyme are listed but the value of this is limited by the fact that neither the concentration of inhibitor, the conditions under which it acts nor the references are usually given. Moreover, the lists are generally far from complete. Eserine is the only inhibitor mentioned for the cholinesterase and 3 M urea the only one for the monamine oxidase.

The book begins with a simple and clear account of the general characteristics and properties of enzymes and ends with a chapter on carbohydrate metabolism and the Szent-Györgyi and Krebs cycles. This chapter includes the facts and theories made familiar by the numerous reviews that have recently appeared on the subject. The book on the whole is clearly and tersely written, well printed, and has only a small number of misprints. Enzyme chemists should find it useful particularly because of the formulae and methods of estimation which are given. Since, as the title implies, chemistry and methods are the main concern of the authors, the book may be considered to have achieved its purpose.

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WILDLIFE REFUGES

Wildlife Refuges. By IRA N. GABRIELSON. New York: The Macmillan Company. 257 pp. 32 plates. 17 figs. 1943. $4.00.

FOR so many years it has been the fate of one who wrote of the wild life of the country to approach the subject with a feeling of despair, that it is quite a relief to find a book which is openly and frankly optimistic, a book which speaks of attainments rather than of defeats and losses. Instead of recording increasing additions to lost causes it contains references to 17,643,915 acres added to the national wildlife refuges and many other acres under private and public ownership. It is indeed heartening to read that "All together they are approaching adequacy," especially as it appears under the name of the director of the Fish and Wildlife Service. Here one may read the history of the movement from the early action by the California legislation in 1870, through a succession of similar events in a series of actions until finally in 1939 332,438 acres were added by the Resettlement Administration. These are all national efforts, to which must be added many more state refuges and numerous private grounds.

The book covers so much ground that it is impossible to note every phase of the treatment, but possibly a brief review of one topic, the Okefenokee swamp area, will serve to present the treatment. It is noted here that this area is impossible to classify, having inhabitants that would permit it to be designated as a migratory wild-fowl area, a big game refuge or a general wildlife refuge. The condition of the forest is much regretted, but it is pointed out that before many years have passed, it will be back in good form again, because of what still remains and because of the rapid growth of vegetation. The prairies are described and also the peculiar methods of travel. Only when he comes to a description of the animal life in the morning and evening of the day does the author's statement that "Okefenokee is marvelous" find justification. A brief general description of the swamp, in which the occurrence of the fabulous "sink holes" is vigorously denied, is followed by an account of the fishing, which is described as "locally famous." It is believed that this is a permanent condition, although the habit of going dry and burning is recognized as a handicap. Finally the author takes up the subject of the future of the swamp, pointing out the desire to restore it to its former state, but also indicating that some animal form such as the panther and ivory-billed woodpecker seem to be entirely absent now. Altogether the account ends most hopefully. Each topic in the book is taken up in a similar way, and the final picture is one of great hope and expectation.

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SPECIAL ARTICLES

INTERFERENCE OF INACTIVE VIRUS WITH THE PROPAGATION OF VIRUS OF INFLUENZA

NOT much is known as to the optimal conditions for the propagation of the influenza A and B virus in the allantoic cavity of the chick embryo. In an attempt to gain this information it was found that the active virus as shown by titration in mice may reach maximal titer (50 per cent. mortality end point) in the allantoic fluid as early as 12 to 18 hours after

infection, depending on the concentration of the inoculum. After attaining maximal concentration the amount of active virus decreased more or less rapidly with further incubation of the embryos until after 3 or 4 days frequently less than 1 per cent. of the highest titer was left.

In contrast to the active virus as titered in mice the red cell agglutinative reaction became positive in the allantoic fluid later and after reaching maximal strength the agglutinating titer remained more or less constant usually for at least 48 to 96 hours of incubation.

Using fluids collected at the height of active virus titer, subcultures ultimately reached equally high red cell agglutinating titers, regardless of whether undiluted or 10,000 to 100,000-fold diluted virus preparations had been used as inocula. However, when allantoic fluids were passed which had been harvested at a time when the active virus titer had decreased markedly, the growth of virus from a concentrated inoculum (0.5 ml of undiluted or 10-fold diluted fluid) was much less than the growth of the virus from an inoculum diluted 1,000-fold or more (Table 1). This

TABLE 1

RELATION BETWEEN THE CONCENTRATION OF THE INOCULUM AND THE PROPAGATION OF THE VIRUS

Inoculum* 0.5 ml diluted	Time of harvest (hours)	Titration in mice LD_{50} per ml† log	Agglutination of chick red cells units per ml
1:10	24	3.3	8
	48	2.0	48
	72	n.t.	48
	96	1.3	16
1:100	24	3.6	0
	48	4.3	224
	72	n.t.	192
	96	2.1	160
1:1000	24	2.0	0
	48	5.8	512
	72	4.5	640
	96	4.0	384

* Lee strain of influenza B.
† 50 per cent. mortality doses per ml.
n.t. = not tested.

paradoxical behavior had previously caused difficulties in the passage of some influenzal strains and even led to their occasional loss.

It appeared possible that inactive virus accumulating with prolonged incubation could interfere on subculture with the propagation of the active virus in the allantoic sac. When active virus in optimal dilution (homologous or heterologous strains) was added to such "inhibiting" fluids and the mixture passed to chick embryos, the resulting formation of the red cell agglutinating agent was never higher than that in other eggs inoculated with a mixture of "inhibiting" fluid and saline solution. The use of normal allantoic fluid and normal chorio-allantoic membrane obtained from embryos of the same age as the "inhibiting" fluid did not prevent the normal propagation of the virus.

In an effort to demonstrate the "inhibiting" factor more clearly, attempts were made to inactivate the viable virus in fluids harvested 24 or 96 hours after inoculation. Heating of the fluids to 56° C. for 30 minutes or even 1 hour, or ultraviolet irradiation[1] under continuous mechanical agitation for 30 to 60 minutes usually inactivated enough of the virus to render them innocuous for mice, but sufficient concentrations of the active agent were left to initiate some multiplication when injected into the allantoic cavity of chick embryos. Although inactivation was incomplete, the following inhibition experiments have given clear-cut results.

Active virus was injected in optimal concentration into embryonated eggs either simultaneously with, or 3 hours following, the administration of the partially inactivated fluids described above, and the propagation of the virus was measured by titration of the allantoic fluids harvested 48 hours later either by inoculation of mice or by red cell agglutination. In each case the titers were reduced to those of the control fluids, prepared by injecting "inhibiting" fluid and saline solution. The experiment summarized in Table 2 shows that no red cell agglutination was noted after 48 hours and further incubation did not alter the result. Titration of the allantoic fluid in mice, on the other hand, revealed the presence of some active virus, which, however, amounted to only a fraction of 1 per cent. of the amount of virus found in the allantoic fluids collected from eggs inoculated with active

TABLE 2

INTERFERENCE OF PARTIALLY INACTIVATED VIRUS WITH THE PROPAGATION OF THE ACTIVE AGENT

First injection 0.5 ml	Second injection 3 hours later 0.5 ml	Results after 48 hours of incubation: Titration in mice LD_{50} per ml log	Red cell agglutinating agent units per ml	Complement fixation antigen units per ml
Irradiated virus* harvested 24 hours after inoculation (undiluted)	saline	4.0	0	0
	virus* 1:1000	4.3	0	0
Irradiated virus* harvested 96 hours after inoculation (undiluted)	saline	2.8	0	10†
	virus 1:1000	3.3	0	10†
Normal allantoic fluid	virus 1:1000	6.8	768	160

* WS strain of influenza A, allantoic fluid.
† Corresponding to originally injected antigen, no formation of antigen.

[1] A Hanovia Examalite quartz lamp was used.

virus following administration of normal allantoic fluid.

Similar interference experiments conducted in mice by the intranasal injection of partially inactivated virus preparations, followed 5 hours later by the active agent, have given results indicating that the same phenomenon may be demonstrated in this species. Protection against as much as 250 50-per-cent.-mortality doses was noted.

Interference of one virus with another has been observed repeatedly. The viruses may be quite unrelated or very closely related, as is the case with neurotropic and non-neurotropic strains of influenza Type A virus.[2] Interference of inactivated bacteriophage with the active agent of the same strain has been observed recently[3] and the present results extend these observations to the influenza viruses. It seems very likely from the data presented that virus having been inactivated during the process of cultivation may cause such interference phenomena and account for the difficulties encountered in the propagation of some strains of influenza virus. A more extensive report will be published elsewhere.

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THE EFFECT OF CASTRATION AND TESTOSTERONE PROPIONATE ON d-AMINO ACID OXIDASE ACTIVITY IN THE MOUSE[1]

IN recent reports from this laboratory we have indicated the effect of castration and testosterone propionate on the activity of three hydrolytic enzymes.[2, 3] We now wish to report findings with respect to an oxidative enzyme, d-amino acid oxidase.

The mice were of an inbred stock, Buffalo-Marsh strain.[4] Castration and implantation of the testosterone propionate[5] pellets were performed when the mice were 18 ± gms body weight. The enzyme activity was determined by a modification of Elvehjem's method. The pyruvic acid formed in the presence of arsenite was determined by the 2,4 dinitrophenylhydrazone method.

The results in Table 1 demonstrate that the mouse kidney loses part of its ability to oxidatively deaminate d-alanine as a result of castration. The administration of testosterone propionate not only restores this property but increases it above normal. These data provide further evidence for our program to elucidate the nature and purpose of the protein anabolic properties of certain steroids originally observed in this laboratory in castrate dogs.[6]

TABLE 1

THE EFFECT OF CASTRATION AND TESTOSTERONE PROPIONATE ON THE d-AMINO ACID OXIDASE ACTIVITY OF MOUSE KIDNEY

Treatment*	No.	Weight of kidneys gms	Pyruvic acid formed			
			Total		Per gram	
			micro-moles	Per cent.	micro-moles	Per cent.
Castrate ..	6	0.259	20.1	−70	75	−54
Normal ...	5	0.402	65.5	..	164	..
Cast. and T. P.	3	0.545	108.0	+64	198	+21
Normal and L. P.	3	0.537	102.0	+55	190	+16

* Body weight at castration 18 ± gms. Treatment for 130 ± days.

The substrates incubated with mouse liver brei in no instance showed the presence of pyruvate. Either the mouse liver possesses no d-amino acid oxidase or it has a different mechanism than the rat for metabolizing pyruvate. We have been able consistently to find pyruvate in good amounts in substrates incubated with rat liver brei according to exactly the same procedure used for the mouse experiments. This difference in enzyme activity between the two species is not too surprising, for we have noted marked differences in the arginase and phosphatase activities in these same species.

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DIFFERENTIAL INHIBITION BETWEEN NORMAL AND TUMOR (CROWN GALL) TISSUE IN BEET ROOTS

A DISTINCT difference has been found by the authors in the action of resorcinol and of cyanide upon the rate of oxygen uptake in the tissues of normal beets and of beet root tumors induced by inoculation with *Phytomonas tumefaciens*.

With normal beet tissue an inhibition of 12 to 14 per cent. is obtained with 0.0166M resorcinol, whereas in tumor tissue this amounts to 20 to 23 per cent.

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5 The testosterone propionate (Perandren) was supplied by the Ciba Pharmaceutical Products, Inc., through the kindness of Dr. E. Oppenheimer.
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