for 10–20 minutes, (*d*) treatment with artificial sea water lacking calcium ion for 1 to 4 hours, or (*e*) irradiation with ultra-violet light of wave-length about 2537 Å for ½ to 3 minutes. Membrane elevation was used as a criterion of activation in *Strongylocentrotus* eggs, dissolution of the germinal vesicle in the eggs of *Urechis*. A small and variable percentage of the eggs of *Urechis* cleaved when activated by any of the artificial treatments. Artificial activation of *Strongylocentrotus* eggs did not result in cleavage.

The addition of 1 mg of acetyl choline bromide to 10 ml of the activating solution before the eggs were placed in the solution resulted, in almost every case, in a marked decrease in the number of eggs activated by the particular treatment. Typical results are presented in Table 1. The addition of 1 mg of physostigmine salicylate to 10 ml of activating solution had effects wholly comparable to those of acetyl choline, except when the eggs were activated with sperm. With the eggs of *Strongylocentrotus* activated by sperm, the only effect noted was a small increase in activation. With the eggs of *Urechis* activated by sperm, a marked decrease similar to that produced by acetyl choline was evident in most cases, but in one experiment an equally marked increase was apparent.

Further analysis of these effects is in progress, and a more detailed account will be published later.

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THE OXYDASE REACTION IN CHICK EMBRYOS AND BROTH MEDIA CONTAINING NEISSERIA[1, 2]

THE value of the oxydase reaction described by Gordon and McLeod[3] for the preliminary identification of meningococci and gonococci has been well established. This reaction is ordinarily elicited by spraying or flooding a petri dish culture with a fresh, one per cent. aqueous solution of either dimethyl or tetramethyl paraphenylene diamine hydrochloride. Colonies of members of the genus Neisseria will then undergo a series of characteristic color changes: pink to maroon to black with the former reagent, and lavender to purple with the latter.

In the course of certain experiments concerned with meningococcic and gonococcic[4, 5] infections of chick embryos, it was found that typical oxydase reactions could be produced upon the infected chorio-allantoic membranes. Forty-eight hours after the inoculation[6] of the chorio-allantoic membranes of eleven-day-old embryos a portion of the shell was removed, the membrane cultured, and a drop of either the dimethyl or tetramethyl paraphenylene diamine hydrochloride solutions deposited thereon. In all instances the Neisseria-infected chorio-allantoic membranes gave positive reactions. Control embryos, either uninfected or infected with organisms other than the Neisseria, yielded negative reactions. Parallel passages on appropriate solid media gave similar findings.

Since the allantoic fluids of Neisseria-infected embryos contain large numbers of organisms, they were subjected to similar tests. This was achieved by overlaying one milliliter of allantoic fluid in a narrow tube with one or two drops of oxydase reagent. The typical sequences of color changes were noted at the interphase. Similar reactions were obtained in broth cultures, including those in the Neisseria fermentation broth previously described.[7]

The specificity of the reaction in both infected allantoic fluid and broth media suggested the possibility that the reacting enzyme was of a soluble char-

TABLE 1

SUMMARY OF OXYDASE REACTIONS OBTAINED IN CHICK EMBRYOS INFECTED WITH VARIOUS AGENTS

Infecting agent	Total number strains	Total number embryos	Chorio-allantoic membrane		Allantoic fluid*		Supernate†	
			Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
N. intracellularis	15	41	18	0	23	0	22‡	1
N. gonorrheae	3	13	12	0	1	0	N.D.§	N.D.
N. catarrhalis	1	2	2	0	N.D.	N.D.	N.D.	N.D.
Staph. aureus	2	2	0	2	N.D.	N.D.	N.D.	N.D.
Sh. alkalescens	1	2	0	2	N.D.	N.D.	N.D.	N.D.
Sh. paradys. Boyd 88	1	2	0	2	N.D.	N.D.	N.D.	N.D.
Paracolon	2	4	0	2	0	2	0	2
P. gallinaceum	1	1	0	1	0	1	N.D.	N.D.
Influenza A virus	1	2	0	2	0	2	0	2
Normal embryos	..	8	0	8	0	1	0	1

* Uncentrifuged.
† Centrifuged, bacteria-free.
‡ The allantoic fluid whose cell-free supernate gave a negative reaction was also negative on culture but morphologically and tinctorially positive for N. intracellularis on smear examination.
§ N.D. = Not done.

[1] With the technical assistance of Frances S. Friedman.
[2] The valuable aid of First Lieutenant (then Sgt.) Barclay R. McGhee, Sn. C., A. U. S., during the early phases of this study is gratefully acknowledged.
[3] J. Gordon and J. W. McLeod, *Jour. Path. and Bact.*, 31: 185–190, 1928.
[4] G. J. Buddingh and A. D. Polk, SCIENCE, 86: 20–21, 1937.
[5] G. Morrow and G. P. Berry, *Jour. Bact.*, 36: 280, 1938.
[6] The surface of a chocolate agar culture which had been incubated for twenty-four hours was scraped and the growth suspended in tryptose-phosphate broth. One drop of this heavy suspension served as the inoculation dose.
[7] D. M. Kuhns and H. A. Feldman, *Am. Jour. Pub. Health*, 33: 1461–65, 1943.

acter. To determine this a number of infected allantoic fluids were centrifuged until the supernates no longer gave positive cultures. With one exception bacteria-free allantoic fluids yielded reactions similar to those obtained prior to centrifugation.

The data are summarized in Table 1.

CONCLUSIONS

(1) Specific oxydase reactions may be obtained with solutions of either dimethyl or tetramethyl paraphenylene diamine hydrochloride when the following are infected with Neisseria: the surface of the chorioallantoic membranes of chick embryos, allantoic fluids both before and after the removal of the infecting bacteria and suitable broth media.

(2) The enzyme responsible for the positive oxydase reaction elicited with the Neisseria is soluble and diffuses into the surrounding liquid environment.

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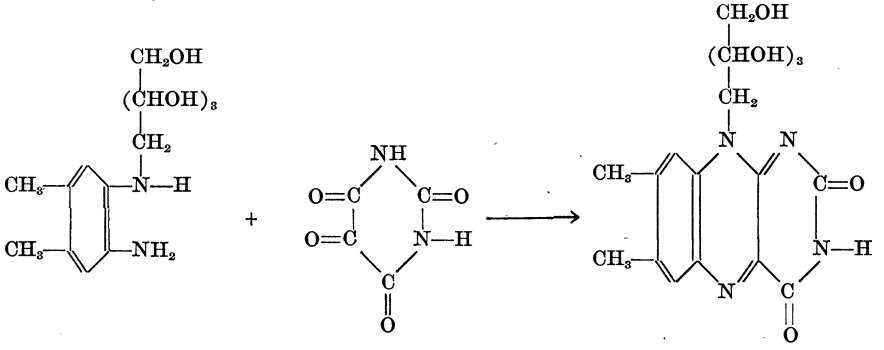
SCIENTIFIC APPARATUS AND LABORATORY METHODS

A MICROBIOLOGICAL AND FLUOROMETRIC TEST FOR MINUTE AMOUNTS OF ALLOXAN[1,2]

THE work of Dunn and coworkers[3,4] has revealed that alloxan administered parenterally produces in rats and rabbits an acute specific necrosis of the islets of Langerhans. This has been amply confirmed in rats, rabbits, dogs and monkeys by various workers.[5–10] The production of experimental diabetes by alloxan and the importance of these experiments has been reviewed by Joslin.[11,12] It has been suggested by Dunn and his coworkers[4] that possible defects in the metabolism of purines or of alloxan in man may play some role in the etiology of diabetes mellitus. For the study of the metabolism of alloxan, a specific method for the determination of alloxan would obviously be advantageous. In the present note, we wish to report our preliminary studies leading to a sensitive and specific test for the quantitative estimation of alloxan in pure solution, which we hope may eventually lead to a specific method applicable to biological materials.



I Alloxan Riboflavin

At the time this work was undertaken, no specific method for alloxan was available. While the work was underway, we learned through private communication from Dr. R. M. Archibald[13] that he had a paper in press which contained several methods for the determination of alloxan. We would like to thank him for his courtesy in allowing us to read his manuscript before it appeared in print. Of those methods described by Archibald the most specific and sensitive is based on the condensation of alloxan with *o*-phenyl-

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[2] The authors also wish to acknowledge with appreciation the technical assistance of Miss Rachel Jewett and Miss Martha Fuchs.

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