the importance of very small amounts of certain minerals, and the ill effects when they are present in excess. These reactions differ in different species; thus, the lemon is considerably more sensitive to boron injury than the orange. This kind of work, likely to be much extended in the future, will throw new light on the distribution of plants.

Dr. Webber's account of the "Cultivated Varieties of Citrus" contains much of general botanical interest. "Two seedlings among the variant types from a lot of approximately a thousand sour orange seedlings were found to lack odor. These two types, which in appearance of foliage and fruit seem mainly to resemble the sour orange, do not produce oil glands and oil. The foliage and the fruit rind when crushed give only the odor of fresh vegetable tissue, not the odor of the oils so characteristic of all members of the citrus family." Presumably this mutation is recessive and it would be possible to breed a distinct type of orange, representing what would be considered a distinct species, or even genus, if found wild. Then there are the blood oranges, which have long been known and are widely cultivated. "It is of interest to note that blood varieties grown in Florida rarely or never show the same intensity and general distribution of color in the fruits as those grown in California." The Washington navel orange, so important in California, is not a success in Florida. The Valencia orange, said to have come from Spain, is "more extensively grown than any other orange in California, Florida, Texas and South Africa, and is doubtless grown more widely and on a larger acreage than any other citrus variety in the world."

Discussions have arisen from time to time, concerning the independence of the scientific worker. He does not like to be regimented. The true solution of such difficulties is to be seen in the book before us. Webber, Swingle and the others did their work according to their best understanding, without external coercion; but no one can deny that for the best results it was necessary for them to cooperate and to have a broad purpose in common. Unquestionably a good deal of scientific work is relatively sterile because done in isolation, without relation to the work of others. Dr. Webber was the ideal man to organize such an enterprise as the all-round study of the citrus problem. His energy and tremendous enthusiasm and his readiness to cooperate with others made this thing possible. As far back as 1892, in Florida, he was associated with Swingle in the study of citrus diseases, and this led to Swingle's botanical work, which made over the whole subject of the citrus allies and added tremendously to our knowledge. Still another factor was the connection with the University of California, making it possible to produce the book in the most excellent and attractive form.

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SPECIAL ARTICLES

AEROSOLIZATION OF PENICILLIN SOLUTIONS[1]

THE use of penicillin as an aerosol in the treatment of infections of the respiratory tract, particularly those caused by pneumococci, staphylococci and streptococci, appears to be feasible for several reasons. Penicillin is known to be bacteriostatic in extremely high dilutions, inhibiting the growth of hemolytic streptococci in quantities as low as .03 micrograms per cc.[2] In addition its activity should not be notably reduced by the organic detritus characteristic of suppurative and pneumonic conditions of the lungs. Since penicillin does not diffuse readily but is rapidly excreted the advantage of local application in all but generalized infections has been stressed by certain investigators.[3] Aerosol inhalational therapy, therefore, appears to be a logical addition to the existing techniques of administering penicillin.

The Long Island Biological Association has conducted a series of experiments to determine if penicillin aerosols can be produced and utilized successfully, using a standard glass nebulizer[4] operated continuously by compressed air. Sodium salt of penicillin was made available by the Committee on Chemotherapeutic and Other Agents of the National Research Council. With one exception we have maintained a concentration of 5,000 Oxford units per cc, using a M/50 phosphate buffer adjusted to a pH of 7. In a desire to conserve penicillin, experiments have not been conducted on an extensive scale.

It is known that the behavior of particulate substances in inspired air is a function of their size; effective penetration of the respiratory bronchioles and alveolae is best attained by small particles. Since rate of air flow through the nebulizing apparatus and physical properties of the solution are two factors influencing particle size, an analysis of buffered penicillin as utilized has been made. Photomicrographic records of penicillin aerosol were made with a modified ultramicroscope. Size determinations, calculated by Stokes Law, showed a distribution illustrated

[1] Aided by a grant from the Josiah Macy Jr. Foundation as part of a project for Chemical Warfare Service.

[2] M. H. Dawson *et al.*, *Ann. Int. Med.*, 19: 707, 1943.

[3] M. E. Florey and H. W. Florey, *Lancet*, 1: 387, 1943.

[4] De Vilbiss 40.

graphically in Fig. 1. The average particle radius was 0.54 μ, with a range of 0.24 μ to 1.18 μ.

Experience with other aerosols led to the conclusion that the penicillin aerosol was of sufficient physical stability and of a size favorable for therapeutic use. However, the chemical stability remained problematical and nothing was known about the fate of penicillin aerosol when inhaled. After preliminary investigation the following conclusions may be made:

(1) As utilized in the nebulizer, penicillin is not altered chemically by the air flow in a manner to

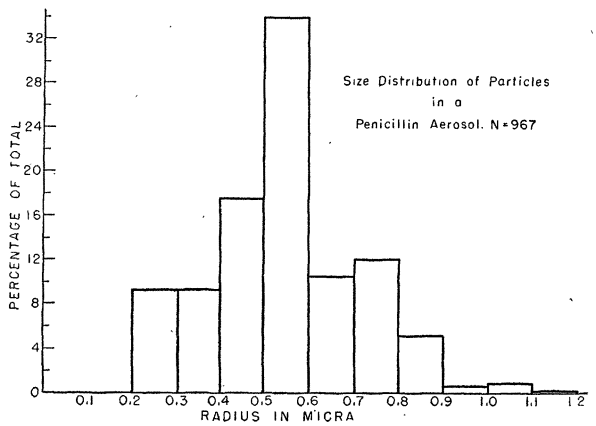

FIG. 1

reduce its potency as determined by the Oxford cup method. This was concluded after the passage of 125 liters of air at 5 liters per minute through a nebulizer containing 5 cc of penicillin solution. A sample from the nebulizer was then compared with a control and showed no loss of potency. An equivalent volume of pure oxygen likewise had no effect in reducing the potency of the solution.

(2) Penicillin forms an aerosol that may be recovered in phosphate buffer with no marked loss of activity. A known weight of penicillin was aerosolized into a suction flask and recovered by washing through phosphate buffer in Milligan gas-washing bottles. The same weight of material was diluted directly in phosphate buffer as a control. An activity loss of about 30 per cent. was observed in three trials. Since the experimental method does not enable one to distinguish between loss of potency and loss of material through inefficiency of the system, a different recovery apparatus was substituted using precooling with dry ice before washing. No significant loss of potency was then found.

(3) Penicillin aerosols penetrate into the lungs. An anesthetized rabbit was exposed to 100,000 units of penicillin aerosol in two hours, with the aid of a modified face mask. It was killed twenty minutes later by percussion. Tracheal perfusion of the lungs with 100 cc of buffer resulted in the recovery of a bacteriostatic substance not found in a control perfusion. Alveolar portions of lung were tested more directly by exposing four mice to 50,000 units of penicillin delivered continuously through a glass cage for one hour. Within the next hour all four animals had been killed, carefully bled, and the lobes of the lungs ground up in 20 cc of buffer and centrifuged. Slight bacteriostatic activity was shown by the experimental supernatant fluid and not by a control.

(4) Penicillin aerosols diffuse into the blood stream. Recovery of penicillin in the urine may be accepted as proof that it has been present in the blood. Two units per cc of penicillin were present in the urine of the experimental rabbit described above. As a further test a simple bottle carburetor with face mask attached was devised for human use, and 50,000 units were aerosolized continuously into the apparatus at 5.5 liters of air per minute and inhaled at 8 liters per minute, including carbureted air. Six per cent. of the total amount of penicillin aerosolized, and probably a larger proportion of the total amount inhaled, was recovered in the human urine within 12 hours.

Although the first lot of penicillin received had a distinct cheesy odor the present allotment, having a potency of 0.8 Oxford units per gamma, is virtually odorless and may be inhaled very comfortably. It should be possible to maintain the blood concentration at a more uniform level using the inhalational method than by intermittent intravenous or intramuscular injection. Between 50 and 80 per cent. of inhaled dust particles of the same size as the penicillin aerosol are retained in the human respiratory tract.[5] Since 0.1 cc of penicillin with a concentration of 250,000 units per cc has been aerosolized and inhaled there appears little doubt that large amounts of penicillin can be introduced by the aerosol method. Probably a saving of penicillin could be achieved by interrupted air flow or by rebreathing, but the final economy can be determined only by weighing therapeutic advantages, against the amount of material required by different techniques of administration. A combination of the nebulizer with an oxygen mask is easily made and compressed oxygen or air may be used to operate the nebulizer either separately or as an adjunct to other forms of inhalational therapy.

Using the oxygen mask-nebulizer combination with a rebreathing bag 3.2 per cent. of 25,000 units aerosolized into the mask was recovered in the urine during the first twelve hours after inhalation. A more economical technique is to place the nebulizer directly in the human subject's mouth for a brief inhalation (15 secs.) followed by breath holding (15 secs.), with a one-half minute interval before repetition. Admit-

[5] A. M. Van Wijh and H. S. Patterson, *Jour. Ind. Hyg. and Tox.*, 22: 31, 1940.

tedly impractical without cooperation the method when properly employed allows recovery of 60 per cent. of the aerosolized penicillin in the urine within twelve hours, comparing favorably with an average recovery of 60 per cent. after intravenous injection.[6]

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ENDOCRINOLOGICAL ASPECTS OF AVIDIN FORMATION IN THE AVIAN OVIDUCT

WE have previously reported that avidin, the antibiotin factor found in egg-white, is formed normally in the oviduct of the hen and that avidin formation may be induced experimentally by the administration of progesterone to immature birds pretreated with stilbestrol.[1, 2] In the present report we wish to present data concerning (1) the ability of steroids other than progesterone to induce avidin production in previously oestrogenized chicks and (2) the quantitative aspects of the oestrogen-progesterone relationship in avidin formation.

Experimental methods, definition of the avidin unit and control data on untreated and stilbestrol treated birds have been previously described.[2] In our earlier studies we have employed chicks approximately six weeks old. We have subsequently found that the day-old chick responds equally well to the same treatment, indicating that no post-hatching reproductive development other than that occurring during the six-day course of stilbestrol conditioning is required for experimental avidin induction. In the experiments reported here we have employed chicks 42 days old with the exception of the testosterone series in which day-old chicks were used.

Both desoxycorticosterone acetate and testosterone propionate induce avidin formation, desoxycorticosterone approximating progesterone in effectiveness (Table 1). Testosterone propionate is effective at a somewhat higher daily dose (3.2 mgms), but its minimal effective dose was not determined.

This lack of specificity in avidin response to the several steroids tested is in keeping with their recognized interchangeability in such other endocrinological reactions as the maintenance of life in adrenalectomized animals and the precipitation of endometrial bleeding in the monkey.[3, 4]

The comparable effectiveness of progesterone and desoxycorticosterone also raises the question of the gonadal or extragonadal origin and of the chemical identity of the steroid normally causing avidin formation in the laying hen.

Table 2 summarizes our data on the latent period required for the appearance of avidin following subcutaneous administration of progesterone with stilbestrol. By the end of two hours avidin is readily demonstrable in the oviduct and relatively high titres are reached within 4 to 8 hours. The latent period for progesterone induction of sexual receptivity in the guinea pig is from 3 to 9 hours, an interval quite comparable with that observed for avidin formation in the chick oviduct.[5]

Since the progestational reaction in mammalian endometria is facilitated by small supplementary dosages of oestrogen but is completely obliterated by relatively large dosages, it seemed desirable to determine the effect of increased oestrogen levels upon the avidin response.[6] The avidin titre is materially elevated when increased oestrogen is administered simul-

TABLE 1

AVIDIN TITRE OF OVIDUCTS OF 6-WEEK-OLD CHICKS, PRETREATED FOR 6 DAYS WITH 0.5 MG. STILBESTROL DAILY; SECONDARY INJECTIONS FOR 2 DAYS THEREAFTER. AUTOPSY CA. 24 HOURS LATER. ALL INJECTIONS SUBCUTANEOUSLY

Secondary injections		Oviducts tested	Avidin titres	
Stilbestrol	DOCA*		Average	Range
mg. daily	*mg. daily*	*No.*	*Units*	*Units*
None	0.05	3	0	.....
"	0.20	4	0.13	0–0.25
"	0.80	4	0.48	0.33–0.60
"	3.20	7	0.56	0.50–0.60
0.5	0.80	4	0.46	0.33–0.50
"	3.20	4	1.89	1.40–2.50
5.0	0.80	4	1.95	2.50–3.30
"	3.20	3	1.28	0.33–2.50
	Progesterone *mg. daily*			
None	0.05	2	0	.....
"	0.20	3	0.17	0–0.30
"	0.80	7	0.42	0.12–0.50
"	3.20	4	1.20	0.60–1.66
0.5	0.05	4	0	.....
"	0.20	4	0.21	0–0.30
"	0.80	8	2.63	1.66–5.00
"	3.20	2	4.40	3.70–5.00
5.0	0.80	4	1.78	1.20–2.50
5.0	3.20	4	3.30	all 3.30
	Testosterone Propionate *mg. daily*			
None	3.20	3+	2.30	1.00–3.30
"	6.40	3+	0.70	0.50–1.00
"	12.80	3+	1.30	1.00–1.60

* DOCA = Desoxycorticosterone acetate.
+ = day-old chicks.

[6] C. H. Rammelkamp and C. S. Keefer, *Jour. Clin. Investigation*, 22: 425, 1943.

[1] R. M. Fraps, R. Hertz and W. H. Sebrell, *Proc. Soc. Exp. Biol. and Med.*, 52: 140, 1943.

[2] R. Hertz, R. M. Fraps and W. H. Sebrell, *Proc. Soc. Exp. Biol. and Med.*, 52: 142, 1943.

[3] R. Gaunt, W. O. Nelson and E. Loomis, *Proc. Soc. Exp. Biol. and Med.*, 39: 319, 1938.

[4] F. L. Hisaw, *Endocrinology*, 33: 39, 1943.

[5] E. W. Dempsey, R. Hertz and W. C. Young, *Am. Jour. Physiol.*, 116: 201, 1936.

[6] F. L. Hisaw and S. Leonard, *Am. Jour. Physiol.*, 92: 574, 1930.