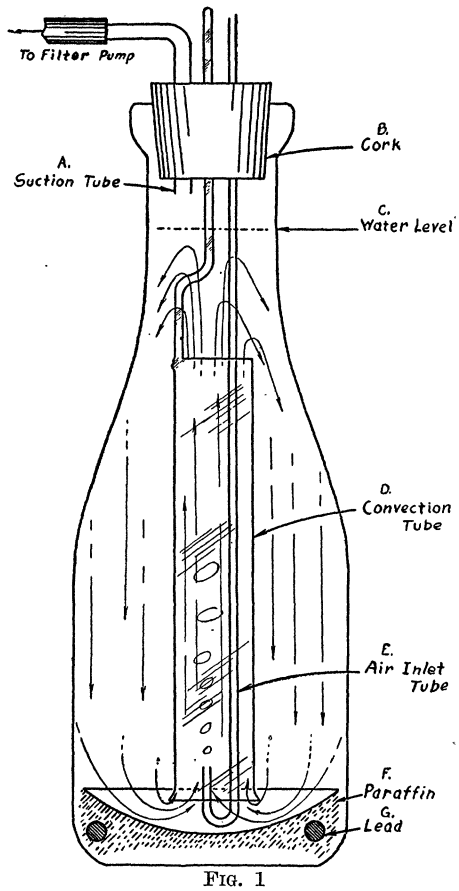

FIG. 1

10 gram pieces of lead are embedded near the lower margin of the paraffin to overcome its tendency to float, in the absence of a perfect seal at the periphery. When cooled it is made cup-shaped with a flame provided by connecting a glass tube, drawn to a tip, to a gas jet. By repeatedly melting the center of the paraffin, and slowly revolving the inclined bottle to allow the melted portion to build up on the sides, the desired cup is obtained. When hard, this can be fire-polished smooth by lightly passing the flame over the surface. The well, thus formed, serves to direct the eggs under the mouth of the convection tube.

The convection tube (D) is made from a 1″ test-tube 6″ long, from the bottom of which 1″ is cut. A $\frac{1}{8}$″ glass rod 6″ long with a $\frac{1}{2}$″ double bend about $\frac{3}{4}$″ from one end is fused by its short section to the cut end of the convection tube, so that when the long section is inserted in the cork the line of suspension is vertical and along the axis of the convection tube. The working distance of this tube is about $\frac{1}{2}$″ above the paraffin, varying with the size of the eggs and the amount of circulation desired. The air inlet tube (E) is $\frac{1}{8}$″ glass tubing 14″ long with a $\frac{3}{8}$″ U bend $\frac{1}{2}$″ from one end. This is suspended inside the convection tube so that the air jet is in the center. The suction tube (A) is $\frac{3}{4}$″ glass tubing 5″ long with a right angle bend in the middle. The cork (B) is drilled in the center to accommodate the convection tube rod, and $\frac{1}{4}$″ along a diameter on each side of the center to accommodate the inlet and suction tubes. The apparatus is set up as shown in the figure (all unions being air-tight) and attached to a filter pump.

When in operation the suction, created by the jet of air passing through the convection tube from the air inlet, will circulate the eggs up through this tube, discharging them at the top; they will then be carried to the bottom of the bottle by gravity and the current, where they will again be drawn up into the convection tube (circulation indicated on figure). The entire medium is filled with moving eggs, which are constantly suspended in the highly oxygenated water. The volume of air passed through, and the relationship of the inlet tube to the bottom of the convection tube, determine the velocity of the current. Dr. Floyd J. Brinley, of this department, has found the apparatus highly successful for the incubation of eggs of the wall-eyed pike (*Stizostedion vitreum*) with respect to percentage and speed of hatching. About 20,000 eggs can be handled in each bottle.

Since the apparatus permits control of temperature, aeration and circulation, thus maintaining a uniform medium under ordinary laboratory conditions, it lends itself readily to many problems involving effects of temperature, chemical solutions, gases, etc., on aquatic organisms. It may be useful in chemical problems utilizing solutions and precipitates. Modifications of the idea also suggest themselves for many problems; for example, the efficiency of the apparatus described by J. Henry Walker, Jr., in SCIENCE for June 26, 1931 (method for oxygenating an aquarium), can be increased by the addition of the convection tube about the air intake. The current created carries air bubbles down into the medium, permitting greater diffusion.

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SPECIAL ARTICLES

THE VAGINAL SMEAR METHOD OF DETERMINING VITAMIN A[1]

IN the quantitative determination of vitamin A,[2] three different biological methods have been used. These are what may be called the growth method, the ophthalmic method, and the vaginal smear method.

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[2] In this paper "vitamin A" refers to all substances which show vitamin A activity.

The growth method, which was the first one used, depends upon the fact that when a young animal is deprived of vitamin A, it ultimately ceases to grow, and when it is fed vitamin A in sufficient amounts, its growth is resumed. The amount of growth obtained under standard conditions is taken as an index of the amount of vitamin A present. The ophthalmic method takes advantage of the fact that when rats are kept on a diet deficient in vitamin A they ultimately develop ophthalmia, which is cured rapidly by the administration of sufficient amounts of vitamin A. The ability or failure to effect a cure, or even the rate of cure under standard conditions, has been used as the quantitative index. The vaginal smear method is based on the observation of Evans,[3,4] who noted that rats on a vitamin A deficient diet showed a persistence of cornified cells in the vagina, and that the addition of large amounts of vitamin A caused a quick resumption of the normal smear picture. He suggested that this fact be used as a method of testing for vitamin A, but he did not compare his results with those obtained by other methods. This was done in 1929 by Coward[5,6] and associates, who reported the vaginal smear method unsatisfactory for general use.

In 1930, however, Schmidt and Schmidt[7] successfully used this method in determining the absorption of vitamin A in the absence of bile. Since then the vitamin A activity of carotin has been established on a quantitative basis, and one gamma (.001 mg) of the same has been accepted as the international unit. By its use, it is now possible to feed constant amounts of active material in all laboratories and to observe the resultant physiological response. We have accordingly compared the sensitivity of the growth method, the ophthalmic method and the vaginal smear method to uniform dosage with carotin as the source of vitamin A.

For our preliminary observations we used rats which had been started on other experiments designed to determine the effect of additions of carotin, egg yolk, liver or burbot oil at various levels to a diet low in vitamin A. These rats were examined by the vaginal smear technique of Evans, and the smear results were compared with the growth of the animal and the rate of cure of ophthalmia. Smears were also run on animals being depleted of their vitamin A stores, and similar comparisons made.

In these experiments, ten animals out of sixty showed persistence of cornified cells in the vagina, in spite of the fact that growth was resumed and ophthalmia was absent or was cured. Most striking was the fact that in none of our animals which had ceased to grow, or which had ophthalmia, were normal smears observed. The greatest difference in results obtained by the different techniques was observed on levels of vitamin A approaching the minimum requirements. However, when the vitamin A dosage was high, or very low, thereby producing respectively rapid cures or none at all, all three methods gave identical results.

Forty-five rats were used for our major experiments. These were placed when 4 weeks old, at 50 to 60 grams in weight, upon a basal synthetic diet low in vitamin A.[8] This diet consisted of casein 18, agar 2, salts 40[9] 4, a yeast rich in vitamin B 6.9, irradiated yeast 0.1 and cooked corn-starch 69.0. The irradiated yeast supplied one Steenbock rat unit[10] of vitamin D per milligram. Smears were examined daily after the vaginal orifice had made its appearance. After cornified cells had persisted for 2 weeks, varying amounts of carotin were added to the diet. The carotin was dissolved in purified cottonseed oil (Wesson oil) and was fed by mouth with a medicine dropper. Animals were weighed thrice weekly. Carotin feeding was continued for 5 weeks and the smears were followed 2 weeks longer.

As the animals were being depleted of their vitamin A reserves, the first sign of the deficiency was a continuation of cornified cells in the vagina, the smear picture otherwise being identical with that of normal oestrum. This phenomenon has been reported by Evans and others. After 2 or 3 weeks, however, a more marked deterioration of the vaginal epithelium manifested itself. The cells became more necrotic with the accumulation first of débris and later of colloidal materials as well. Occasionally leucocytes reappeared, sometimes in large numbers, but the colloidal cloudy material present rendered it relatively easy to distinguish between these smears and those of normal dioestrum. Time and again typical cornified cells appeared at intervals, as well as combinations of cornified cells and leucocytes. In other words, the vaginal smear of a vitamin A deficient rat at first revealed a picture of a mass of persisting typical cornified cells and later a mass of débris, alternating in variable sequence with leucocytes plus débris, cornified cells plus débris, true cornified cells or leucocytes plus

[3] H. M. Evans and E. S. Bishop, *Jour. Med. Res.*, 1, 335, 1922.
[4] H. M. Evans, *Jour. Biol. Chem.*, 77, 651, 1928.
[5] K. H. Coward, *Jour. Physiol.*, 67, 26, 1929.
[6] K. H. Coward, B. G. E. Morgan, F. J. Dyer, *Jour. Physiol.*, 69, 349, 1930.
[7] W. Schmidt and C. L. A. Schmidt, *Univ. Calif. Pub. Phys.*, 7, 211, 1930.
[8] H. Steenbock, M. T. Nelson and A. Black, *Jour. Biol. Chem.*, 62, 275, 1924.
[9] H. Steenbock and E. M. Nelson, *Jour. Biol. Chem.*, 56, 355, 1923.
[10] H. Steenbock, S. W. F. Kletzien and J. G. Halpin, *Jour. Biol. Chem.*, 97, 249, 1932.

cornified cells. This latter appearance, if the deficiency remained uncorrected, lasted until death. Either or both of these types of smears were designated as "low A smears."

In rats placed on a low A diet at 50 to 60 grams and at an age of 22 to 25 days, the vaginal orifice usually makes its appearance in our colony at an age of 39 to 84 days—the average being 53 days. Within this range, no correlation was found between the age at maturity and the height of the animal at the beginning of the experiment. But it was impossible to use all our female rats for the vaginal smear-growth-ophthalmia correlations because the vaginal orifice frequently was not established by the time growth had ceased or ophthalmia had developed. As a matter of fact, out of one group of 45 rats on the low A diet, 9 remained sexually immature during the course of the experiment and 3 matured after cessation of growth.

Rats on the low A diet generally showed "low A smears" before the appearance of other symptoms of the deficiency, although in some cases all symptoms appeared at about the same time. However, in no case were normal smears observed after the appearance of other low A symptoms. Again referring to the previously mentioned group of 45 rats, 22 per cent. showed low A smears and stopped growing at the same time, 18 per cent. showed low A smears 3 to 7 days before stoppage of growth, and 33 per cent. showed low A smears 7 to 14 days before cessation of growth. As far as ophthalmia and growth are concerned, the majority of our rats stop growing at the time the ophthalmia makes its appearance and *vice versa*.

The different symptoms of vitamin A deficiency were found to respond unequally to carotin administration, Table 1. On 3 to 5 γ carotin per day the general tendency was for the ophthalmia to be cured and slow growth to continue for five weeks without restoration of the normal smear picture. The addition of 10 γ or more of carotin per rat per day restored dioestrous smears within a week, and oestrum in two weeks.

As a result of our experience we have come to the conclusion that the vaginal smear method can be used as a quantitative method for the determination of vitamin A. It has the following desirable features:

(1) The response to the addition of vitamin A is rapid —generally within a week.

(2) The amount of vitamin A necessary for a positive response is definite.

(3) Animals of various ages, even older animals as discards from other experiments, can be used and one animal may even be used repeatedly.

(4) Like the cure of ophthalmia the reaction is specific, but unlike ophthalmia the reaction is not so complicated and, therefore, more reliable. Needless to say it is far more specific than growth.

(5) The test can be used to determine vitamin A sufficiency in experiments with adult females without introducing complications.

TABLE I

COMPARATIVE CORRECTION OF SYMPTOMS OF VITAMIN A DEPLETION BY DIFFERENT AMOUNTS OF CAROTIN

Carotin given daily	Lot	No. of rats	Growth in 5 weeks	Condition of eyes after 5 weeks	Condition of smears after 5 weeks	Remarks
gamma			*gms.*			
3	179	4	15	Cured	Not cured	Slight swelling of eyelids cured in 10 days
		2	22	"		Immature
5	180	4	27	—	Not cured	
		1	23	—	Cured	Normal smears restored in 4 days
	173	2	35	Cured	Not cured	Swelling of eyelids cured in 1 week
		1	40	—	Cured	Normal smears restored in 4 days
10	174	1	40	Cured	"	Low A smears for 23 days before cure—eyes purulent when carotin was added
		5	35	"	"	2 rats showed swelling and bleeding of eyelids; cured in 1 week—Normal smears within 1–7 days
	176	1	25	—	Not cured	Low A smears reappeared after third week
		3	41	Cured	—	Matured 2 weeks after carotin addition . . . smears normal
20	175	4	41	—	Cured	Normal smears restored in 2–4 days
	177	6	36	Cured	"	Normal smears restored in 1–8 days —swelling of eyelids cured within a week
40	178	4	50	"	"	Normal smears restored in 1–4 days

The following features operate against its general use:

(1) It requires considerable routine work.

(2) Only female rats can be used.

(3) Many females fail to mature sexually on a vitamin A deficient diet before they succumb to other symptoms and therefore can not be used.

(4) It is less sensitive than growth or the cure of ophthalmia for the detection of small amounts of vitamin A, *i.e.*, it takes more carotin to restore normal smears than to restore growth or to cure ophthalmia. For quantitative work this objection is not serious, since the amount of carotin (10 to 20 γ) necessary for normal smears is quite as definite as the amount (5 to 10 γ) necessary for growth, or the amount of (3 to 5 γ) necessary for a cure of ophthalmia.

(5) Like the ophthalmic method it is difficult to express the results in numerical terms. In this respect growth or maintenance in weight has obvious advantages, if one can be certain that all dietary requirements other than vitamin A have been supplied by the basal ration.

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CHEMICAL CHANGES IN THE BLOOD IN ADDISON'S DISEASE[1]

THE significance of the loss of inorganic base from the body in diabetic acidosis, in cholera and in diarrhoeas of infancy, in high intestinal obstruction and in terminal chronic nephritis is now thoroughly appreciated. All these pathological states are characterized clinically by severe prostration, dehydration, lowering of the serum chloride concentration, nitrogen retention and frequently by a decrease in the bicarbonate concentration of the blood and "shock." Since these changes are also typical of the disease picture of severe adrenal insufficiency, we have studied in detail the electrolyte structure of the blood in three patients suffering from Addison's disease.

In the accompanying table it will be seen that all three patients showed deviations from the normal which were qualitatively similar. Of particular interest is the decrease in total base which occurs entirely at the expense of sodium, because in all three cases the potassium content of the serum was either at a "high normal" level or definitely increased. The changes observed in chloride, bicarbonate, non-protein nitrogen and blood sugar concentrations are those already well known. The retention of inorganic sulphate probably accompanies that of non-protein nitrogen. The abnormalities of the blood found in our patients suffering from Addison's disease are similar to the unpublished results attained by Zwemer in his study of adrenalectomized cats.

The loss of sodium and the increase in potassium in the blood serum of the patient E. C. is striking. This patient received two injections of extract of adrenal cortex prepared by Dr. R. Zwemer before the blood study was made and two injections later, but died in spite of this therapy. The patient J. V. was well except for increasing pigmentation of the body and buccal mucous membranes and for occasional vomiting spells for four months preceding admission to the hospital. In this patient, the decrease in the serum sodium concentration is also definite, but less marked than in the case of E. C. The blood of the third patient, M. W., was examined on three occasions. On admission the patient was markedly prostrated and, as in the other cases, showed a significant decrease in

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BLOOD CHANGES IN ADDISON'S DISEASE

Name	Date	T.B.	Na	K	Ca	Cl	HCO_3	PO_4	SO_4	Prot.	NPN	Sugar	Haemato-crit	Blood pressure	Remarks
		me.q. p.l.	me.q. p.l.	me.q. p.l.	me.q. p.l.	me.q. p.l.	me.q. p.l.	me.q. p.l.	me.q. p.l.	me.q. p.l.	mgm. per 100 cc	gm. p.l.			
E. C.	5– 9–32	127.7	109.5	8.74	6.6	70.1	18.0	4.3	4.0	18.2	75.9	0.72	44.4	76/52	Received 4 injections of Adrenal Cortex (Zwemer). Died on 5–11–32.
J. V.	5–17–32	144.9	131.3	5.3	5.4	97.6	26.1	1.9		17.0	32.0	0.91	41.7	102/60	
M. W.	7–19–32	136.3	123.5	5.3	5.3	88.6	21.8	2.6	2.3	15.1	39.0	0.73	42.0	85/55	
M. W.	7–26–32	125.8	107.8	7.1	4.8	72.7	21.5	2.4		16.9	45.0	0.80		65–70/48	Received 10 cc. of Eschatin on 7–26.
M. W.	8– 2–32	147.5	133.0	5.1	5.9	93.8	27.5	2.6		13.5	20.6	1.20	31.4	84/60	Patient had received 12–15 gm NaCl daily for 6 days.