

of .2 to .5 cc into the yolk sacs of other embryos. Embryos of various ages were used and transfers were made at various intervals following inoculation.

The morphology of the microorganism varies depending somewhat more upon the age of the embryo from which the smear is made than upon the duration of the infectious process. Cultures from early generations showed mixtures of encapsulated and non-encapsulated forms. As passages increased smears from older embryos showed predominantly unencapsulated forms while smears from young embryos showed a predominance of encapsulated ones. Experience has determined that inoculation of 5- or 6-day embryos into the yolk yields consistently in 72 hours a rich culture that is almost wholly encapsulated. The encapsulated form has been maintained in series. This form inoculated into 12-day embryos grows out largely unencapsulated. Embryos from 1-through 13-days incubation support subsequent development of infection following inoculation into the yolk sac. Our experience indicates that the yolk of every embryo inoculated (700-800) has yielded a growing culture.

This microorganism grows evidently extracellularly in the yolk of the embryo. Smears and histological sections also show that it occurs both in its encapsulated and unencapsulated form inside epithelial cells of the yolk sac membrane, also within mononuclear cells of inflammatory exudate in the yolk and its sac. Notwithstanding direct inoculation evidence that it grows on the chorioallantois or invades the embryo proper from the yolk sac is as yet lacking. Inoculation into the amniotic fluid of the intact embryo seems to support growth feebly.

Infected yolk of the 10th passage was drawn from the embryo and stored in sealed test tubes at 5° C, 25° C, 37° C and at -78° C. After 17 days stored yolk from the first three groups was diluted 50 per cent. with .85 per cent. NaCl and injected in .5 cc amounts into 6-day yolk sacs. That stored at 25° C grew out promptly in 72 hours; that stored at 5° C and at 37° C grew slowly, but all embryos showed a good growth at the end of a week. Similar tests for survival made at the end of 33 days showed that the microorganism survived only at 25° C. Yolk stored at -78° C has not yet been tested for survival of the organism.

Mice inoculated intraperitoneally showed no evidence of infection. Dogs inoculated intra- and subcutaneously have not yet shown evidence of infection. *Macacus rhesus* monkeys were inoculated intra- and sub-dermally. Organisms resembling Donovan microorganisms were demonstrated by smears from nodules that persisted for 4 days, but the nodules regressed and have shown no further activity.

The fact that the microorganism appeared to grow in the yolk of the intact developing embryo made its culture in that medium *in vitro* seem feasible. Yolk alone from uninfected 5- and 6-day embryos in test-tubes did not support growth, but with the addition of bits of embryonic chick heart it gave a fairly good culture in 6 days at 37° C. After 2 serial passages in yolk-heart medium a subculture in yolk without heart was initiated. Strains have thus been maintained through ten serial passages *in vitro* during 7 weeks in yolk with and without heart.

Experiments are in progress at the present time to determine the relationship of this microorganism to the human infection, granuloma inguinale. A series of experiments to determine something of its antigenic relation to the disease is also being carried out. More detailed consideration of its morphological, cultural, antigenic and pathogenic characteristics will be the subject of further study.

KATHERINE ANDERSON

DEPARTMENT OF PATHOLOGY,
VANDERBILT UNIVERSITY MEDICAL SCHOOL,
NASHVILLE, TENN.

EFFECTIVENESS OF VITAMIN A IN THE TREATMENT OF DEFECTIVE COLOR VISION

SEVERAL reports have appeared in this journal during the past year on problems of color-blindness, tests for color sensitivity and the value of vitamin A as a remedial agent in conditions of defective color perception.^{1, 2, 3, 4} The first suggestion that vitamin A could be used with effect in cases of impaired sensitivity to color was made in a report by Dunlap and Loken⁵ before the Southern Society for Philosophy and Psychology. This was followed by the statement that cases were "cleared up" with vitamin A in from three to eight weeks, using doses of 25,000 units per day.¹ Later, these writers stated that 80 per cent. of their cases were able, after vitamin A treatment, to pass chart tests which they had failed previously.³

The practical importance of color vision has increased greatly since the beginning of the war. With well over a million men of draft age showing some degree of color deficiency, the possibility of salvaging even a small percentage of this man-power for the armed services or for vital work in industry was certain to attract attention.

Present knowledge of function of the visual receptors, plus the fact of a demonstrable hereditary

¹ K. Dunlap and R. D. Loken, *SCIENCE*, 95: 2474, 554, May 29, 1942.

² E. MURRAY, *SCIENCE*, 96: 2484, 133-5, August 7, 1942.

³ K. Dunlap and R. D. Loken, *SCIENCE*, 96: 2489, 251-2, September 11, 1942.

⁴ E. MURRAY, *SCIENCE*, 96: 2498, 448, November 13, 1942.

⁵ K. Dunlap and R. D. Loken, *Psychol. Bull.*, 39: 585, October, 1942.

determinant and a tendency for color thresholds to remain constant under varied environmental conditions, indicated that the defect probably would not respond to vitamin treatment. However, until just ten years ago, we were not fully aware of the intimate and essential role of vitamin A in the normal function of the rods. The discovery of the relationship between vitamin A and rhodopsin could suggest that it might also be required in some similar but unknown way by the cones.

Following the first report by Dunlap and Loken, some preliminary observations were made on 16 college students who had defective color vision. Most of them had failed to pass tests of the Army Air Corps, but were quite anxious to do so. Three tests were used with this group: the Ishihara, the American Optical Company's pseudoisochromatic plates and the Westcott lantern slide, which is a modification of the yarn test principle for the purpose of group testing. Vitamin A (as purchased locally in the form of concentrated fish oil) was given to these subjects in doses of 25,000 units daily for eight weeks or more. One of this group took 250,000 units daily on prescription of a local physician. Fourteen of these cases, including the one just mentioned, showed no improvement, but two of them finally achieved almost perfect scores. Both of these subjects subsequently passed the Army Air Corps tests and are now training in that service.

Had all these preliminary tests been negative, it is unlikely that further observations would have been made, but it seemed difficult at the time to account for the improved performance of these two individuals except as being a result of vitamin A treatment. In the light of the results reported below, it may be necessary to accept another hypothesis. It should be said, however, that the original defect in these two cases was of slight degree.

In order to check the possibility that some benefit could be derived from vitamin A by a few individuals, some extensive observations were made under more rigid conditions. Group tests of 897 R.O.T.C. freshman cadets at Louisiana State University showed 65 who had various degrees of weakness in color sensitivity. Individual tests were then given to 58 subjects who began taking 50,000 units of vitamin A on alternate days. This schedule was continued for eight weeks. After having taken 1,400,000 units, each subject was retested under the same conditions as before.

Because there are many reasons why subjects, especially those who are not volunteers, might fail to follow instructions for taking vitamin A, it was considered important that this part of the test be carefully supervised. Accordingly, the subjects were required to swallow the capsules at regular hours at a dispensing station.

No significant improvement in color sensitivity was shown by any individual in the group of 41 who finished the eight weeks period of treatment. Most of the records of response to the 62 plates of the American Optical and Ishihara tests were practically identical before and after taking vitamin A. The maximum improvement shown by any individual was a correction of three previous errors. The lantern slide test gave essentially the same results, although there was more variability in the responses. An analysis of the reliability of these tests and their value as a convenient means of detecting color "blindness" will be presented in a later report.

The procedure in the present experiment differed from that of Dunlap and Loken in a few respects. We used a large number of subjects of approximately the same age (median, 17 years, 9 months) and living under very similar conditions throughout the period of testing. We are able to assert positively that all subjects actually took vitamin A, because this was done regularly in the presence of the experimenter or an associate. The material used was a vitamin A ester of high potency, determined spectrographically and confirmed by bio-assay.

It may be concluded from these tests that vitamin A in doses of 25,000 I.U. daily for eight weeks fails to produce any significant improvement in color sensitivity. It seems improbable that administration of the vitamin for longer periods of time would change this result, although observations are being continued on several subjects.

Murray⁴ warns against the unfortunate consequences which could follow acceptance of vitamin cures for color deficiency until the permanency of results is thoroughly tested. The present study does not entirely dispose of the possibility that a few men, perhaps with minor color vision defects, may improve slightly; but the number who could use vitamin A for this purpose is so small as to be negligible. We need not, therefore, be concerned about the numbers who can pass the test temporarily.

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J. H. ELDER

LOUISIANA STATE UNIVERSITY

VITAMINS IN DEHYDRATED SEEDS AND SPROUTS

THE common use of sprouted seeds in the diets of oriental peoples appears to rest on a sound nutritional basis, if we are to judge by the vitamin content of such food materials. It has already been reported that significant increases in the concentration of riboflavin, nicotinic acid and biotin occur during germination of