This request was refused, and the experiments were therefore terminated. The refusal was based upon the contention that unless preliminary experiments were undertaken to test the effects of optimum volume and concentration of H 11 upon M 63 and C 3H tumours the results would be valueless. After considerable thought had been given to this suggestion it was decided that no useful purpose would be served by undertaking dosage experiments.

Summary

Evidence is presented that grafted Twort mammary carcinoma is not a satisfactory tumour upon which to test a chemotherapeutic substance for growth-inhibitory properties. This statement is based upon extensive attempts to propagate this tumour, which often does not take and has a high regression rate (Table I).

No growth-inhibiting effect of H 11 could be demonstrated in experiments in which grafted Twort carcinoma was the test tumour (Table II) nor in experiments in which a grafted spindle-cell sarcoma in homologous mice was tested (Table III).

H 11 had no effect on the growth rate of spontaneous mammary cancer in inbred mice as compared with that of similar untreated tumours (Table IV). New tumours were not prevented from arising, and metastasis took place in spite of the treatment.

Grateful acknowledgment is due to Dr. E. C. Armstrong and Dr. W. J. Martin: to the former for much help in propagating the grafted tumours and in administering the H 11 treatment; to the latter for the statistical analysis of the experiments.

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DEFICIENCY OF VITAMIN A IN UNIVERSITY STUDENTS

BY

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In spite of the intense current interest in the nutritional state of the people of this country there are few positive data from which it is possible to draw any very definite conclusion (see *Lancet*, 1948). It might therefore be of interest to describe the results of a recent investigation which suggests that about one-fifth of a group of university women students are deficient in vitamin A.

Dark-adaptation and the Effect of Vitamin A

This investigation was carried out in February and March, 1948, and consisted in the determination of the dark-adaptation of a group of students and the study of the effect upon it of vitamin A. The dark-adaptation was measured by the Crookes adaptometer (Goddard, 1945). The principle of construction and the technique of use of this apparatus are similar to those of the Haines apparatus employed in previous studies (see Yudkin, 1945). The complete dark-adaptation curve was determined twice for each subject and the second curve taken as representing the true course of dark-adaptation, since it has been shown both in this and in previous investigations that any improvement due to a "learning factor" is almost, if not quite, completed in the second test.

Some of the students were then given three capsules containing 100,000 i.u. of vitamin A daily for 14 days, and others were given capsules containing arachis oil for the same time. The dark-adaptation was measured about 24

hours after the last dose by one of us (S.D.), who did not know which students had received the vitamin A or, indeed, the proportion of these students.

It has been shown that an improvement in dark-adaptation is always accompanied by a decrease in the "final" rod threshold (Yudkin, Robertson, and Yudkin, 1943). We have taken as a significant improvement a decrease in this threshold of more than 0.15 log $\mu\mu\text{l}$. (micromicrolamberts). None of the 19 control subjects in this investigation showed any improvement after the administration of the capsules containing arachis oil. Of the 46 subjects given vitamin A, seven showed a significant improvement of dark-adaptation. Of a smaller series of six postgraduate students given 200,000 i.u. of the vitamin daily for eight days, two showed a significant improvement (Table I).

TABLE I.—Effect of Vitamin A on the "Final" Rod Threshold of 52 Students

Effect of Vitamin A on Threshold	No. of Students
Increase of 0–0.15 log $\mu\mu\text{l}$.	3
No change	13
Decrease of 0–0.15 log $\mu\mu\text{l}$	27
Decrease of more than 0.15 log $\mu\mu\text{l}$ —i.e., significant improvement	9

Dietary Intake of Vitamin A

The studies of dark-adaptation suggest that nearly one-fifth of the students were deficient in vitamin A. It is of interest, then, to know something of their dietary intake of the vitamin. It has not been possible to determine this for all the students whose dark-adaptation has been measured, but we have some information about the diets of other students, both resident and non-resident, of the same college.

It is the practice here for the postgraduate dietetic students, who are not in residence, to record by weighing their own intake of food for one week in October. Records of 14 students were kept in 1946 and 1947. The composition of the foods eaten was assessed by reference to the tables (M.R.C., 1945a). The values for carotene were divided by three, the commonly accepted factor, in order to convert them into equivalents of vitamin A. Of the 14 students, three had an average daily intake of less than 2,500 i.u. of vitamin A, seven an intake of between 2,500 and 5,000 i.u., and four an intake of more than 5,000 i.u.

The dietary intakes were also recorded of 20 first-year residential students during November, 1947. The data were collected by dietetic students for an investigation carried out by Dr. Magnus Pyke and Miss K. Chamberlain, of the Ministry of Food. The total amount of food consumed by the students was recorded for a week by the "matching" method, and in addition the food of three meals each day, comprising about one-half of the total intake, was recorded by the "weighing" method. Since the latter method is the more accurate we have assessed the intake of vitamin A by a combination of the two methods, adding the intake of the three meals as determined by the weighing method to that of the rest of the food as determined by the matching method. Of the 20 students, four had an average daily intake below 2,500 i.u. daily, 14 an intake between 2,500 and 5,000 i.u., and two an intake of more than 5,000 i.u. Table II summarizes the dietary intake of vitamin A for the total of 34 students of both groups.

TABLE II.—Average Daily Intake of Vitamin A by 34 Students

Average Daily Intake	No. of Students
Below 2,500 i.u.	7
Between 2,500 and 5,000 i.u.	21
More than 5,000 i.u.	6

There is reason to believe that 2,500 i.u. of vitamin A represents, as nearly as can be ascertained, the average minimal daily requirements of the vitamin for young adults. It is of interest, therefore, that the proportion of students whose consumption of vitamin A was below 2,500 i.u. daily is very similar to the proportion whose dark-adaptation improved with vitamin A (7 out of 34, or 21%, and 9 out of 52, or 17%).

Since nearly all the preformed vitamin A in the diet is derived from rationed foods—butter, margarine, cheese, and milk—it is interesting to see how much vitamin was consumed by the students. At the times of the surveys the rations of these foods contained between 1,000 and 1,100 i.u. of the vitamin daily. Of the 34 students whose diets were examined, the intake of preformed vitamin A from these foods was between 700 i.u. and 4,000 i.u. daily—mean 1,700 i.u. Thirty of the students consumed more than the 1,100 i.u. daily to which their rations entitled them. We do not wish to comment on this beyond pointing out the relevance of such findings to recent rather heated discussions which attempt to evaluate accurately the probable intake of nutrients from a study of the foods theoretically available from the rations.

The remainder of the vitamin A consumed was almost entirely in the form of the provitamin carotene. Assuming again that carotene has one-third of the activity of preformed vitamin A, the intake was between 750 and 4,000 i.u. daily—mean 1,850 i.u. The proportion of the preformed vitamin A to the total intake was between 22% and 79%. It is perhaps not surprising that the range of intake of vitamin A and of carotene was larger in the group of non-resident students (Table III).

TABLE III.—Average Daily Intake of Preformed Vitamin A and of Carotene

Group	Total A (i.u.)	Preformed A (i.u.)	Carotene (i.u.)	Proportion of Preformed A
20 residents ..	1,650–4,900 (3,450)	700–2,950 (1,600)	950–3,000 (1,850)	31–67% (46%)
14 non-residents ..	2,300–6,650 (3,750)	900–4,000 (1,900)	750–4,000 (1,850)	22–79% (51%)
Total ..	1,650–6,650 (3,550)	700–4,000 (1,700)	750–4,000 (1,850)	22–79% (48%)

Correlation of Dark-adaptation and Intake of Vitamin A

Thirteen of the students took part in the two investigations described, so that data are available for each of them both of their intake of the vitamin and of the effect of vitamin A upon their dark-adaptation. Three of these had intakes of vitamin A of less than 2,500 i.u. daily; each of these three, and no others, showed a significant improvement of dark-adaptation following administration of vitamin A (Table IV).

TABLE IV.—Correlation between "Vitamin-A Labile" Dark-adaptation and Dietary Intake of Vitamin A

Subject	Daily Intake of Vitamin A (i.u.)	Effect of Supplements of Vitamin A on Dark-adaptation
47	6,650	—
42	5,650	—
5	4,900	—
4	4,350	—
19	4,050	—
46	3,750	—
44	3,450	—
9	3,050	—
16	3,050	—
6	3,000	—
45	2,450	+
11	2,400	+
43	2,300	+

+ Indicates a decrease of more than 0.15 log μ l. in the final rod threshold. Subjects 42–47 were non-resident; the remainder were resident in college.

Discussion

Requirements of Vitamin A

It should be stressed at once that the determination of the requirements of any nutrient is a much more complex matter than is usually realized. There has been a tendency to suppose that it is possible to stipulate a definite amount of a nutrient, a consumption below this amount leading inevitably to a state of deficiency and above it to a state of optimal nutrition in respect of that nutrient. This attitude is quite unrealistic for many reasons, which have been discussed in some detail elsewhere (Yudkin, 1948). Two aspects in particular concern us here. The first is that there is undoubtedly with many nutrients, and probably with all, a range of intake between the minimal, which is necessary to prevent frank deficiency disease, and the optimal, beyond which no improvement in any respect will occur. Whereas it may be comparatively easy to determine the former, since below it obvious signs will develop, it may be extremely difficult to determine the latter, since increasing amounts of the nutrient may produce such ill-defined effects as increased resistance to infection or increased general well-being.

The second important difficulty in determining requirements is that there are differences between various individuals even of the same sex and age, and also differences in the same individual at different times. One cannot therefore suggest an amount of a nutrient as the requirement for, say, young women and assume that any one of them consuming less than this would necessarily be receiving an insufficiency and any one consuming more would necessarily be receiving a sufficiency.

With these considerations in mind, we must conclude that it is only within fairly wide limits that we can ascertain what the requirements of vitamin A are for such subjects as we have examined. First, if we know just how much vitamin A will prevent a deterioration of dark-adaptation we cannot assume that this amount will be adequate for all other physiological functions. Secondly, if we find that 2,500 i.u. of vitamin A daily will just prevent a deterioration of dark-adaptation in one individual it is more than likely that higher or lower amounts will be necessary for other individuals.

An examination of the published work confirms these conclusions. Callison and her colleagues studied in young adults the effect on dark-adaptation of diets deficient in vitamin A, to which was then added vitamin A or carotene (Booher and Callison, 1939; Booher, Callison, and Hewston, 1939; Callison and Orent-Keiles, 1947). The carotene was given either as the pure provitamin in oil or in its natural state in vegetables. The amount of vitamin A required to restore normal dark-adaptation was between 1,300 and 3,900 i.u. (mean 2,400) and of carotene in oil between 2,200 and 7,200 i.u. (mean 4,300) daily for five subjects. They found that carotene was less well utilized from oily solution than from spinach and peas, but better than from carrots.

A subcommittee of the Medical Research Council (M.R.C., 1945b) reported that in three subjects 1,300 i.u. of vitamin A or 2,600 i.u. of carotene in oil daily restored normal dark-adaptation of subjects on a diet deficient in the vitamins. Unlike the American workers, they found that carotene is less well absorbed and hence presumably less well utilized from spinach, as well as from cabbage and carrots, than it is from oily solution.

Since the subjects in these investigations were on special diets and it is known that other dietary components affect requirements, and since it is commonly held that, in

general, carotene is not so effectively absorbed from vegetables as from solutions in oil, it is likely that more vitamin A is needed in normal diets than the amounts suggested by the M.R.C. subcommittee.

Our results show that, in a small number of subjects, dark-adaptation was apparently normal in 10 subjects consuming more than 3,000 i.u. of vitamin A daily and abnormal in three consuming less than 2,500 i.u. It should be emphasized that these figures include variable amounts of carotene, which has been assumed to have an activity in the human being of one-third that of vitamin A. Since this value is not accurately known, but varies both with the subject and with different types of food, and since the number of subjects is small, we do not wish to overestimate the importance of our findings in this respect. We would only justify this discussion about the relevance of our findings to the requirements of vitamin A by pointing out that, unlike previous work, our data refer to subjects on a normal diet, and that such correlative studies of physiological function and dietary intake clearly offer a most important means of gaining knowledge of requirements. We hope it will be possible to carry out observations of this nature on a much more extensive scale.

Previous Studies

It would have been of great interest if we had been able to compare the data presented here with similar evidence of deficiency of vitamin A obtained in previous years. There exists unfortunately very little work of a similar sort referring to conditions in this country. Most of the work which does exist relates to estimations of vitamin A in the blood or in the liver, where standards of normality are not yet agreed. As regards dark-adaptation, very few previous reports include studies of the effect of vitamin A. In a series of 335 factory workers in whom only the "final" rod threshold was measured, the proportion which showed improvement after supplementation with 5,000 i.u. daily for eight months was significantly greater than the proportion in the control subjects—73 out of 147 (50%) compared with 58 out of 188 (31%) (Yudkin, 1944). In 320 school-children, on the other hand, studies involving determination of "final" rod threshold or of the complete course of dark-adaptation showed that no improvement followed the administration of vitamin A (Robertson and Yudkin, 1944). In a small series of adults, studied intensively in the way described in the present report, six out of a total of 14 given vitamin A or carotene showed an improvement in dark-adaptation in which the "final" rod threshold decreased by more than $0.15 \log \mu\text{l}$. (Yudkin, Robertson, and Yudkin, 1943). In another small series of 15 Army officers, studied in the same way in the winter of 1944–5 by one of us (J.Y.), none showed a significant change in the course of dark-adaptation following large supplements of vitamin A. This may well have been due to the appreciably higher amount of vitamin A in Army rations than in civilian rations. For example, the chief sources of preformed vitamin A—butter, margarine, cheese, and milk—supplied between 1,500 and 1,600 i.u. daily in the Army rations and between 1,000 and 1,100 i.u. daily in the civilian rations.

It seems then, that, on the basis of improvement of dark-adaptation following administration of vitamin A, deficiency of the vitamin among civilian adults is not a new phenomenon. It is unfortunately impossible to answer adequately the important question whether there has been any increase or decrease in the proportion with this deficiency. This of course does not make it any the less disturbing that as many as one-fifth of a sample of young adult women should show deficiency of vitamin A at the present time.

Significance of Impaired Dark-adaptation

There are two attitudes one might adopt towards the findings that about one-fifth of our subjects have a dark-adaptation which improves with vitamin A. One is that, since dark-adaptation is a normal function of the body, any impairment in it which can be improved by vitamin A must be taken as indicating that the physiological demands of the vitamin have not been met. It is indeed possible that other functions not so readily investigated are also affected. Moreover, even if the intake of the vitamin is sufficient just to restore dark-adaptation, one cannot assume that such other functions are also restored. The second attitude one might adopt is that dark-adaptation is not a very important physiological function anyway, so that a slight impairment is of little practical consequence and need not cause any concern about the adequacy of the diet. We can say at once that we believe this attitude to be quite untenable. We would stress that most of the methods which are used to determine the probable state of nutrition of individuals, such as the determination of levels of nutrients in the blood or in the urine, involve many assumptions in their interpretation; here, on the other hand, we are concerned with the measurement of a normal physiological activity of the body. If we find that such an activity is in one-fifth of a population adversely affected by the diets they consume we must consider that the situation is far from satisfactory.

Summary

The course of dark-adaptation of 52 women university students was measured and the effect of supplements of vitamin A observed. Nine showed a significant improvement of dark-adaptation—that is, a decrease of more than $0.15 \log \mu\text{l}$. in the "final" rod threshold.

A study of the dietary intakes of 34 students showed that the average daily intake of seven of them was below the probable average requirement of 2,500 i.u. daily. Thus both the measurement of dark-adaptation and the assessment of the dietary intake of the vitamin reveal a similar proportion of subjects (about 20%) who were probably consuming a diet deficient in vitamin A.

Of the 13 students for whom measurements of both dark-adaptation and dietary intake were available the three whose average daily intake of vitamin A was below 2,500 i.u. were the only three whose dark-adaptation improved with vitamin A.

It is impossible to say whether the proportion of deficient subjects has been influenced by recent dietary restrictions, but the fact that something like 20% of women university students are deficient in vitamin A is one which cannot be regarded lightly.

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Dr. D. J. A. Alban-Jones has been awarded scholarships by the Belgian and Netherlands Governments through the British Council to study obstetrics and gynaecology. He will work at the Hôpital St. Pierre in Brussels for eight months and then go to Amsterdam in April, 1949, to take up the Netherlands scholarship for four months.