FURTHER STUDIES IN ARSOPHENAMINE HYPERSENSITIVENESS
IN GUINEA PIGS

IV. VITAMIN A (CAROTENE) IN RELATION TO THE SENSITIZATION OF
GUINEA PIGS TO OLD ARSOPHENAMINE

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Investigations of R. L. Mayer and Sulzberger have demonstrated that diet has a significant influence on sensitization of guinea pigs to arsphenamine. Since the problem of which specific dietary factor produces the influence has not been entirely solved, and since the solution of this problem might also be of importance in the prevention and therapy of arsphenamine disturbances in man, further examinations on this subject were made. A brief historical survey of the questions which are involved precedes the report of the present experiments.

HISTORY

Frei, (1a), as well as Nathan and Munk, (2), obtained specific sensitization of man by intracutaneous injections of arsphenamines. Frei, (1b), and Sulzberger, (3a, b, c), achieved the same results in guinea pigs with a like procedure.

Sulzberger (3d, e, f) observed a high degree of variations in the responses of guinea pigs to this type of sensitization, and, with his co-workers, examined the factors accounting for these variations and fluctuations. R. L. Mayer and Sulzberger (3d) stated that a very important factor was the composition of the diet: guinea pigs receiving green summer fodder were highly resistant to sensitization to neoarsphenamine (and paraphenylenediamine) whereas those receiving dry winter fodder were highly susceptible. They also discussed the question as to whether these differences in the effect of fodder were due to vitamins or to acid-base balance.

In examining this question from the point of view of vitamins, Sulzberger and Oser (3g) found that sensitization of guinea pigs to neoarsphenamine was possible in a fairly high percentage of animals when medium amounts of crystalline Vitamin C were added to a scorbutogenic diet, whereas sensitization was difficult in animals fed very high and very low Vitamin C doses. These findings were confirmed by Cormia, (4a, b), who expressed the opinion that the inhibitory effect of high Vitamin C doses on the arsphenamine hypersensitivity of guinea pigs consisted less in a prevention of sensitization than in a suppression

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of specific reactions (also see Chapman and Morrell (5), M. B. Cohen (6), discussed by Cor-
mia (4a, b) and by Sulzberger (4b)). Recently, in discussing a report by Cormia, Sulz-
berger (4b) emphasized that the effect produced by the quantitative variation in Vitamin C
content was not so marked as to make him believe that Vitamin C could account for the
results previously found in his experiments with winter and summer fodder.

In the same discussion, Williamson (4b) mentioned that he had had a great deal of diffi-
culty in sensitizing guinea pigs to neoarsphenamine when these animals were deprived of
Vitamins A plus D, and that reactions in sensitization also became difficult to elicit when
the animals were on diets low in Vitamins A plus D. Prior to the time of these remarks,
injections of Vitamin A plus D were used by Dainow (8) in man for curing and desensitizing
occupational dermatitis (see also Hellerström (9)).

GENERAL CONSIDERATIONS

At the laboratory of J. Jadassohn’s Clinic in Breslau, where the experiments of
R. L. Mayer and Sulzberger were performed, the summer fodder of guinea pigs
consisted, according to these investigators and to the personal knowledge of the
present author, of fresh young grass plus a small proportion of a mixture of
mashed potatoes and bran. The winter fodder was composed of field cured hay,
beets, and larger proportions of the potato-bran mixture with addition of dry
oats. Both summer and winter fodder were rich, and not deficient in any way.
With both types of fodder guinea pigs were healthy and gained regularly in
weight. The summer fodder was alkalizing, the winter fodder acidifying (see
R. L. Mayer and Sulzberger). Both feeds differed in other respects also, but
most conspicuously in their vitamin content.

Fresh young grass, which was the dominant ingredient of the summer fodder,
contains abundant amounts of numerous vitamins. In the process of field cur-
ing and transforming grass into hay (which was used during the winter months),
several of the vitamins are considerably diminished. A further loss may take
place in the storage of hay. In the case of such vitamins as thiamin, and some
other members of the Vitamin B complex, and of Vitamin E, a possible loss of
vitamin value in the winter fodder (if a loss of these vitamins occurs) probably
was compensated for by the feeding of additional grains which are rich in the
aforementioned vitamins. In the case of Vitamin D content, the winter fodder
might have been even more potent than the summer fodder, since fresh grass is
entirely devoid of this vitamin and acquires it by the process of sun curing.

The present article deals with some experiments on the influence of Vitamin A
on specific hypersensitivity of guinea pigs to old arsphenamine. Vitamin A, like
Vitamin C, belongs to those vitamins which are most prominent in fresh grasses,
and which lose much of their activity by natural drying, and a certain part also
by storage. Grasses, like other plants, contain this vitamin in the form of its

3 See McDonald and Johnson (7), who, however, did not succeed in producing definite
and indisputable sensitization to neoarsphenamine in their guinea pigs.

4 For details see W. R. Graham, Jr., G. O. Kohler, and C. F. Schnabel (10).

5 For details of the vitamin content of human and animal food under varying conditions
see tables of E. P. Daniel and H. E. Munsell (11); M. A. Boas Fixsen and M. H. Roscoe (12);
and L. E. Booher and E. R. Hartzler (thiamin) (13). See also the booklet “The Vitamins"
of the American Medical Association (14).

6 There are only few data available on the effect of drying and storage on these vitamins.
precursor carotene. In a first experiment the question was examined as to whether Vitamin A had any influence on the process of sensitization of the skin of guinea pigs to old arsphenamine; and in a second experiment, as to whether there was any influence of this vitamin on the development of specific skin reactions in animals previously sensitized to old arsphenamine. Finally, the surviving guinea pigs of both series were tested for their anaphylactic sensitivity to old arsphenamine. The animals of the first experiment received amounts of Vitamin A in four grades, ranging from very high doses to very low doses, and including doses comparable to "summer fodder" and to "winter fodder." In the second experiment the effect of very high and very low Vitamin A amounts were compared. Vitamin A was given as carotene either in the form of natural foodstuff or in pure form, dissolved in cottonseed oil. As a control, the same amount of the same brand of plain cottonseed oil was fed.

**EXPERIMENTAL DATA**

**Experiment I**

Thirty-eight white virgin guinea pigs, weighing from 300 to 350 grams each, were kept on the regular basal diet of this laboratory, "Purina complete rabbit chow", a mixed dry foodstuff which was also used by M. B. Cohen (6) and by McDonald and Johnson (7) in their guinea pig experiments on the influence of Vitamin C on sensitization to neoarsphenamine. It contained sufficient amounts of calories, minerals and vitamins, with the exception of Vitamin C (for details see H. J. Smith (15)). In its Vitamin A content, the foodstuff after storage may be compared with the "winter fodder" of the experiments of R. L. Mayer and Sulzberger. Vitamin C and water were supplied by daily addition of the vitamin A free inner leaves of stored white mature cabbage. Under these feeding conditions the guinea pigs gained regularly in weight and preserved a glossy coat.

After 40 days the guinea pigs were divided into five groups. Group one, "carotene group," (9 animals) received, in addition to the previous diet—chow plus white cabbage—excessive amounts of Smaco Carotene in (cottonseed) oil; for 16 days each animal was given 0.6 cc., equal to about 4000 international vitamin A units daily, and after that 0.5 cc. every other day. The solution was applied by mouth with a syringe before feeding. Group two, "cottonseed oil group," (8 animals) received, instead of carotene plus cottonseed oil, the same amount of the same brand (S.M.A.) of plain cottonseed oil. Group three, "carrots group," (8 animals) received, in addition to the same diet—chow plus white cabbage—abundant daily amounts of fresh mature carrots. Group four, "plain diet group," (6 animals) received the same diet—chow plus white cabbage—without further addition. Groups one to four continued gaining in weight under these feeding conditions.

A fifth group, "oats group," (7 animals) was placed on a vitamin A free diet consisting of oats and the inner leaves of stored white mature cabbage. These animals stopped gaining in weight. Two of them died within three weeks, one of the two with symptoms suspicious of vitamin C deficiency. Therefore, in this group, and concurrently in all the others, the stored white mature cabbage (see above) of the diet was replaced by the inner etiolated leaves of fresh green immature cabbage as a richer vitamin C and water source. Under the new regimen which, however, was, for group five, no longer entirely devoid, but now poor in vitamin A, the remaining five animals of this group also gained for a while until there was a second standstill after about 7 to 9 weeks of oats feeding. Two more animals of this group died after having been on oats for 5½ and 7½ weeks respectively, one with severe anemia and the other after an extreme weakness of the forelegs. Changes of the eyes were not observed.

According to "New and Nonofficial Remedies," 1939, Smaco Carotene in Oil is prepared by dissolving in cottonseed oil carotene-Smaco with an extract of carrots.
The remaining three animals survived. On all dead animals of *group five*, and on two normal controls, *histological examinations* of the uterus and renal pelvis were done. There were no pathologic changes in these organs, in both control animals and in the animals which had died after 3 and 5½ weeks. In the uterus of the animal which had died after 7½ weeks, scattered foci of a metaplastic, stratified epithelium with cornification were found (see fig. 1), whereas the renal pelvis did not display definite changes.

After twenty days of the aforementioned special diets, the animals of all five groups received, on the left flank, the *first intracutaneous injection* of 0.1 cc. of a solution of 0.15 gm. old arsphenamine (Winthrop) in 100 cc. saline. Six to nine days later, flare-ups appeared in all nine animals of the “carotene group,” in all eight animals of the “carrots group,” in six of the eight of the “cottonseed oil group,” in four of the six of the “plain diet group,” and in three of the five surviving of the “oats group.” The flare-ups in the animals of the “carotene” and “carrot groups” seemed to be on the average slightly stronger than those of the other three groups. Four weeks after the first injection, all animals, still on the same diets, received on the right flank an *intracutaneous reinjection* of the same brand and concentration of old arsphenamine as used for the first application. With few exceptions, which were distributed without clear preference among the individuals of the various groups, the reinjections revealed hypersensitivity of all groups to old arsphenamine. The impression again arose that the reactions of the “carotene” and “carrot groups” were, on the average, slightly stronger than those of the others; but the differences were not conclusive.

**EXPERIMENT II**

Eighteen white virgin guinea pigs, weighing 350 to 450 grams each, were kept on a diet consisting of “Purina complete rabbit chow” and the inner leaves of stored white mature cabbage (see Exp. 1). After 38 days each animal received, on the left flank, an intracutaneous injection of 0.1 cc. of a solution of 0.15 gm. old arsphenamine (Winthrop) in 100 cc. saline. Sixteen of the eighteen animals showed flare-ups. When reinjected on the right flank with the same amount of the same brand of old arsphenamine four weeks later, these sixteen gave positive, and the two others, negative skin reactions. All eighteen animals were then put on a *vitamin A poor diet* consisting of oats and the inner etiolated leaves of green young cabbage (see Exp. 1). At the same time each of nine of the eighteen, (eight with positive and one with negative reactions) was given 0.5 cc. *Smaco carotene in cottonseed oil*, equal to about 3500 international vitamin A units daily by mouth before feeding (“carotene group”). The nine others were given the same amount of the same brand of *plain cottonseed oil* (“cottonseed oil group”). Both groups gained in weight for the next five weeks, after which there was no further gain in the group fed plain cottonseed oil. After 25 days of this diet all eighteen animals received, on the left flank, a *second reinjection* of the same kind as before. The reactions did not differ from those following the first reinjection, and the reactions of the “carotene plus cottonseed oil group” were on the average of the same strength as those of the plain “cottonseed oil group.” One animal of the plain “cottonseed oil group” died after 7 weeks of vitamin A poor diet. It had large amounts of abdominal fat (see Wolbach and Howe (16)), fat infiltration of the liver, and in the uterus, foci of metaplastic stratified epithelium with beginning keratinization (see fig. 2). The renal pelvis did not show definite changes.

*Anaphylactic responses of the guinea pigs of Experiments I and II*

Three to four weeks after the intracutaneous reinjection of old arsphenamine, each of the surviving guinea pigs of Experiment I and II, while still on the special diet, was injected with 5 mg. of un-neutralized old arsphenamine (Winthrop) in the jugular vein. This amount was dissolved in one cc. saline solution prepared from triple distilled water. To non-sensitized control animals the dose of 5 mg. proved to be innocuous. In the sensitized animals few anaphylactic reactions were obtained, and no conspicuous differences among the various diet groups were observed (see table 1). At the end of this experiment the remaining guinea pigs were put on the regular fodder of the laboratory.
Discussion of Experimental Conditions and Results

Since in this investigation vitamin A did not appear to be an important factor in influencing sensitization of guinea pigs to old arsphenamine, a brief discussion of the experimental conditions will suffice. In Experiment I, the basal diet of four of the five groups of guinea pigs consisted of a commercially prepared, dry foodstuff which according to the manufacturer, contained about 450 vitamin A units.

Fig. 1. Uterus Horn of Guinea Pig Fed on Vitamin A Poor Diet for 7½ Weeks
Note focus of metaplastic stratified epithelium with keratinization. (Experiment I, group V.)

Fig. 2. Uterus Horn of Guinea Pig Fed on Vitamin A Poor Diet for 7 Weeks
Note foci of metaplastic stratified epithelium with slight keratinization. (Experiment II, group II.)
units of carotene per 100 grams (15). The inner etiolated cabbage leaves which were added to this foodstuff as a vitamin C and water source did not increase the vitamin A content of the fodder considerably (11, 18). In comparison with the 10,000 and more vitamin A units found in 100 grams of fresh young grass, the vitamin A content of this diet could be considered as approximately equivalent to that of the “winter fodder” of the experiments of R. L. Mayer and Sulzberger. One group of guinea pigs received this diet in combination with plain cottonseed oil which is usually devoid of vitamins after refinery (19), and another received it without further addition. The “summer fodder” was represented by the same diet enriched in one series by large amounts of fresh mature carrots as vitamin A source, and in another, by excessive doses of carotene in cottonseed oil. Carotene was chosen because it is the natural vitamin A source of herbivorous animals.

### TABLE 1

**Anaphylactic responses of guinea pigs of Experiments I and II***

<table>
<thead>
<tr>
<th>EXPERIMENT NUMBER</th>
<th>DIET GROUP</th>
<th>NUMBER OF ANIMALS</th>
<th>DEATH†</th>
<th>SEVERE SYMPTOMS‡</th>
<th>SLIGHT SYMPTOMS§</th>
<th>NO SYMPTOMS‖</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Carotene</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Carrots</td>
<td>8</td>
<td></td>
<td>1</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cottonseed oil</td>
<td>8</td>
<td></td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plain diet</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oats</td>
<td>3</td>
<td></td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Carotene</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Cottonseed oil</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

* Five non-sensitized control animals, kept on plain diet, did not respond to the test dose of 5 mg. A sixth control animal, receiving 10 mg, developed severe shock.
† Death after severe cramps within 3 to 5 minutes; on autopsy: lungs inflated, heart beating.
‡ Severe cramps, falling on side, voiding, severe drop in temperature.
§ Slight cramps.
‖ Questionable responses (isolated mild symptoms as coughing, scratching, drop in temperature of more than two degrees C) are also included in this column.

and, at the same time, is a very innocuous substance even in excessive doses (20). It is known that guinea pigs need relatively large amounts of carotene in comparison with rats (21).

The fifth group of guinea pigs of Experiment I received first a vitamin A free diet; and later, when this diet proved to be deficient also in other directions, a vitamin A poor fodder consisting of oats and the inner etiolated leaves of green young cabbage. The vitamin A deficiency of this fodder was demonstrated by histological changes found in an animal which died after a feeding period of 7½ weeks (16, 17). The uterus of this animal contained scattered foci of metaplastic stratified epithelium with keratinization (see fig. 1). As far as is known, no other condition except vitamin A deficiency produces such changes. Histological changes of the uterus during oestrus (which might be suspected), would not conform to the changes produced by vitamin A deficiency (22, 23). There was only
the question as to whether the guinea pigs of group five were already in a vitamin A poor state when the sensitizing arsphenamine injection was applied, which was after a feeding period of 20 days (see protocol). However, according to Bessey and Wolbach (24), guinea pigs retain very little vitamin A even when fed on diets rich on carotene—in contrast to rats which may store enough vitamin A to supply their nutritional needs for several months. It should also be noted that in the experiments of R. L. Mayer and Sulzberger on summer and winter fodder, a feeding period of 8 to 12 days preceding the arsphenamine sensitization, was sufficient for changing the susceptibility in either direction.

In Experiment II, all animals were fed the same vitamin A poor diet as in group five of Experiment I, namely, oats and inner etiolated leaves of green young cabbage. In addition, half of the guinea pigs received excessive doses of carotene in cottonseed oil, and the other half the same amount of plain cottonseed oil. One of the animals of the latter group which died after 7 weeks of feeding on this diet, showed characteristic foci of metaplastic stratified epithelium of the uterus with beginning cornification (see fig. 2). In this experiment, the intracutaneous arsphenamine tests were applied after a feeding period of 25 days.

In the anaphylaxia tests which were performed in all surviving animals three to four weeks after intracutaneous testing, while the guinea pigs were still on their special diets, the responses were less pronounced than in former experiments of Frei and Sulzberger (1c). This was perhaps due to the fact that in the present experiment the solutions were made up without admixture of guinea pig serum (see Landsteiner and Jacobs (25)).

The results of the first experiment can be interpreted as follows: A comparison between guinea pigs fed on vitamin A (carotene) rich diets, and others receiving medium vitamin A doses, did not show significant differences in their responses to intracutaneous sensitization to old arsphenamine and to subsequent intracutaneous reinjection. The impression prevailed that the responses of the groups receiving medium doses were slightly weaker than those on high doses. Homologous results were obtained in guinea pigs kept on a vitamin A deficient diet. Here, however, the number of surviving animals was too small for definite conclusions. These results are similar to recent observations of Williamson (4b) on the influence of vitamin A plus D on sensitization of guinea pigs to neoarsphenamine, although in the present experiment the differences were less pronounced.

In the second experiment, groups of guinea pigs, first sensitized and tested to old arsphenamine and then put on very high and very low vitamin A (carotene) doses respectively, reacted to intracutaneous injections of old arsphenamine in an identical way, and, at the same time, with equal strength as they did before changing the diet. In the anaphylaxia test, the surviving animals of both experiments, still on their special diets, did not show conspicuous differences in their responses to intrajugular injections of moderate doses of old arsphenamine. However, it is still unknown as to whether the influence of winter and summer fodder respectively, also extends to the anaphylactic type of arsphenamine hypersensitivity. Accessory findings to the present experiments were that excessive amounts of cottonseed oil, rich in certain unsaturated fatty acids (especially
linoleic acid; see Burr and Burr (26); Jamieson (19)) did not influence the sensitization of guinea pigs to old arsphenamine. Neither did large amounts of oats, with their content of thiamin and other members of the vitamin B complex, of vitamin E, and of supposed "toxic factors."

It is difficult to draw exact conclusions from these experiments about the work of R. L. Mayer and Sulzberger because of the fact that the present author employed old arsphenamine, whereas R. L. Mayer and Sulzberger used neoarsphenamine. However, these experiments did not furnish any evidence that the high vitamin A (carotene) level of summer fodder had anything to do with the strong resistance of guinea pigs to sensitization to neoarsphenamine while on the summer feed diet, and conversely, that the low vitamin A (carotene) level of winter fodder had anything to do with the high susceptibility of guinea pigs to that sensitization while on the winter feed diet.

SUMMARY

Variations of the vitamin A (carotene) content of the fodder within a wide range, had no significant influence on intracutaneous sensitization of guinea pigs to old arsphenamine. The impression prevailed that animals on vitamin A rich diets gave on the average slightly stronger skin responses to the sensitizing injection, and the following reinjection, than those on moderate or deficient doses.

Groups of guinea pigs first sensitized and tested to old arsphenamine and later put on very high and very low vitamin A (carotene) doses respectively, reacted to intracutaneous injections of old arsphenamine in an identical way, and with the same strength as they did before changing the diet.

Variations of the vitamin A (carotene) content of the fodder did not influence the anaphylactic responses to old arsphenamine in animals which were sensitized while receiving their special diets, or in others which received these diets after sensitization.

Excessive amounts of cottonseed oil, rich in linoleic acid, or great quantities of dry oats rich in thiamin and some other members of the vitamin B complex and in vitamin E, did not influence the sensitization of guinea pigs to old arsphenamine or the reactions of previously sensitized animals.

These experiments (performed with old arsphenamine) did not furnish any evidence that variations of the vitamin A (carotene) content of the diets were responsible for the differences in the effect of winter and summer fodder in sensitization of guinea pigs to neoarsphenamine.

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