crease would be paralleled by an increase in antigenic potency, tests in guinea pigs showed that the digestion with trypsin at either 37° or 45° resulted in a marked loss of potency. These data also confirmed the stability of the measles immunogen at 45° (without trypsin) reported previously(11).

In relation to the purification and concentration of measles virus, it is clear that the CF property of measles virus preparations should not be used to evaluate such procedures without frequent animal potency tests.

Summary. Digestion of whole measles fluids with crystalline trypsin at either 37° or 45° results in a 2- to 3-fold increase in amount of sedimentable complement fixing antigen. This treatment, however, markedly reduces the ability of these fluids to induce formation of neutralizing or complement fixing antibodies when injected in guinea pigs.

1. Charney, J., Fisher, W. P., Machlowitz, R. A., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 601.
2. Smadel, J. E., Wall, M. J., *J. Exp. Med.*, 1937, v66, 325.
3. Weil, M. L., Warren, J., Breese, S. S., Jr., Russ, S. B., Jeffries, H., *J. Bact.*, 1952, v63, 99.
4. Crocker, T. T., *J. Immunol.*, 1956, v77, 1.
5. Schwerdt, C. E., Schaffer, F. L., *Virology*, 1956, v2, 665.
6. Gresser, I., Enders, J. F., *ibid.*, 1961, v13, 420.
7. Frankel, J. W., Cooke, H., Deviney, J. T., Warner, M., West, M. K., *Abst. Bact. Proc.*, 1958, 58.
8. Fogh, J., Lund, R. O., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 532.
9. Osler, A. G., Strauss, J. H., Mayer, M. M., *Am. J. Syph., Gon. & Ven. Dis.*, 1952, v36, 140.
10. Johnson, M. J., *J. Biol. Chem.*, 1941, v137, 375.
11. Dewitt, C. W., Nook, M. A., *J. Immunol.*, 1960, v84, 194.

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A Salmonellosis Resistance Factor For the Guinea Pig.* (26981)

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A relationship between nutritional status and resistance to disease has long been recognized but the nature of this relationship remains obscure(1). Schneider(2) observed decreased mortality among mice infected with *Salmonella enteriditis* when a purified basal diet was supplemented with whole wheat, malted barley or dried egg white. Mortality among guinea pigs subjected to whole-body X-radiation was significantly reduced when the animals consumed raw cabbage or broccoli(3), but it is not clear whether or not the increased survival was the result of increased resistance to infection. Preliminary observations made in our guinea pig colony suggested that consumption of raw cabbage decreased mortality due to salmonellosis. To test this effect under controlled conditions guinea pigs fed a purified diet, with and without cabbage supplementation, were artificially infected and the observations are reported here.

Methods. Female guinea pigs were reared from weaning on the purified basal diet described below or on the basal supplemented with cabbage or cabbage fractions. In so far as possible they were continued on the same diets during the experimental period, but in all cases were fed the experimental diets for at least 2 weeks prior to infection.

Two groups of animals, 10-16 weeks of age and weighing 400 to 600 g, were used in each of 5 trials. They were kept in cages with raised wire floors and housed in a room maintained at 75 ± 5°F. All received a basal purified diet and one-half received in addition a daily supplement of cabbage, *ad libitum*. The basal diet contained, in percent, washed casein 30.0, sucrose 43.3, wood pulp 15.0, salts(4) 4.0, soybean oil 4.0, po-

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TABLE. I. Cabbage supplementation and resistance to salmonellosis

Observations	Basal diet	Cabbage supplement
Mortality,%	77(81)[1]	44(88)
Avg. Survival Time, days	18.6(62)	20.6(39)
Survivorship, ST_{50}[2]	19	38+
Spleen Wt/Body Wt × 100	.46(55)	.41(63)

1. No. of observations upon which value is based.
2. ST_{50} is no. of days until 50% of group is dead.

tassium acetate 2.7, magnesium oxide 0.5, DL-methionine 0.2, ascorbic acid 0.2, choline Cl 0.1, chlortetracycline HCl 0.0025 and the vitamin supplement previously described(5). Chlortetracycline protects guinea pigs against certain infections but does not prevent salmonellosis(6). It was included in the diet in an attempt to reduce secondary infections.

After the animals were adjusted to the experimental conditions they were infected with *Salmonella typhimurium* by intraperitoneal injection of approximately 100,000 organisms. The animals were then weighed and observed daily for a 42-day period. The Salmonella organism was originally isolated from a naturally infected guinea pig and identified serologically. The inoculum was prepared by growing the organism, which had been stored on beads in a frozen state, in trypticase broth overnight, collecting by centrifugation and resuspending in sterile saline solution (0.15 M).

Results. A summary of the results of 5 trials is shown in Table 1. The mortality rate among animals fed the basal diet varied from 64 to 100% and averaged 77%. Variation among the trials was not greater than might be expected due to chance ($X^2 = 1.24$; $P>0.8$). Mortality among the cabbage supplemented animals ranged from 27 to 54% and averaged 44% ($X^2 = 2.16$, $P>0.7$). The difference in mortality due to cabbage supplementation (33%) was highly significant ($X^2 = 18.2$, $P<0.01$). There was no significant difference in mean survival time of the animals that died but the time required for one-half of the animals to die (ST_{50}) was more than twice as long for the supplemented group. Since in all but one trial (23 days) 50% of the animals fed cabbage did not die during the 42-day experimental period it is not possible to assign an accurate ST_{50} for this group. It can be stated that the period averaged greater than 38 days. Spleens and livers of all infected animals were fibrotic, greatly enlarged and contained viable organisms.

In one trial animals that survived the experimental period were carried for a total of 5 months. Of those fed the basal diet 50% survived and only one-fourth showed gross evidence of spleen or liver damage. Of the supplemented group 75% survived, and one-half showed organ damage. Thus, it appears that while the cabbage supplement increased the resistance of the animals it did not prevent or decrease the gross tissue damage caused by the infection.

Clearly cabbage supplementation of guinea pigs fed the basal diet decreased mortality rate, but neither the identity of the factor supplied nor the mechanism of its action is known. The basal diet contains adequate quantities of all the recognized vitamins and amino acids known to be required by the guinea pig, including an abundant supplement of ascorbic acid. When raw cabbage was supplied *ad libitum*, guinea pigs consumed about 25% of dry matter from cabbage and the remainder from the basal diet. Cabbage dry matter contains approximately 20% of crude protein and 2.5% of sulfur amino acids. Thus, substitution of cabbage for basal diet would tend to decrease protein but slightly increase sulfur amino acids. In growth trials carried out along with this study and to be reported later, additional methionine did not stimulate growth rate whereas cabbage supplementation did. For these reasons, cabbage is believed to contain an unrecognized nutrient required by the guinea pig.

Summary. Guinea pigs fed a purified diet with and without *ad libitum* supplementation with cabbage were inoculated intraperitoneally with *Salmonella typhimurium*. Over a 6-week period the supplement decreased mortality significantly and more than doubled survivorship. There was no effect on tissue damage among survivors. An unrecognized nutrient that increases resistance to salmonellosis is postulated.

1. Scrimshaw, N. S., Taylor, C. E., Gordon, J. E., *Am. J. Med. Sci.*, 1959, v237, 367.
2. Schneider, H. A., *Ann. N. Y. Acad. Sci.*, 1956, v66, 25, 337.
3. Spector, H., Calloway, D., PROC. SOC. EXP. BIOL. MED., 1959, v100, 405.
4. O'Dell, B. L., Morris, E. R., Regan, W. O., *J. Nutrition*, 1960, v70, 103.
5. ———, Vandepopuliere, J. M., Morris, E. R., Hogan, A. G., PROC. SOC. EXP. BIOL. MED., 1956, v91, 220.
6. ———, Regan, W. O., Hogan, A. G., *ibid.*, 1957, v96, 553.

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Anti-estrus Drugs on Subestrus of Ovariectomized C_3H Mice.* (26982)

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Gonadectomy in certain strains of mice leads, in time, to development of tumors of the adrenal cortex which secrete estrogen and/or androgen(1,2 and others). There is considerable evidence that these tumors develop as a result of the increased secretion of gonadotropin following castration. Nodular hyperplasia of the adrenal cortex of an intact mouse may be produced by parabiosis with a spayed partner(3). Estrogen, as well as certain other steroids(4), and hypophysectomy(5) will prevent their occurrence. Cortisone, in amounts sufficient to cause adrenal atrophy, does not prevent their development(6).

That formation of adrenal tumors and secretion of sex hormones from them may be dependent upon different mechanisms is shown by the findings that caloric restriction (7), thiourea administration(8) and unilateral adrenalectomy(9) prevent the appearance of subestrus but not the evolution of adrenal tumors. Yet secretion of estrogen by adrenal tumors may also be controlled by gonadotropin as evidenced by the observation that when an estrogen-secreting tumorous mouse is parabiosed with an intact mouse diestrus develops in the tumorous mouse, supposedly due to preferential utilization of gonadotropin by the ovaries of the intact mouse, but when a tumorous mouse is parabiosed with an ovariectomized mouse increased cornification in the vaginal smear of the tumorous mouse occurs due, supposedly, to the increased gonadotropin from the recently ovariectomized mouse acting on the adrenal tumor of its partner(10). On the other hand, administration of gonadotropin does not induce subestrus in tumorous mice on caloric restriction(11).

Inasmuch as reserpine, chlorpromazine, meprobamate(12), nidroxyzone(13), perphenazine and Enheptin† (2-amino-5-nitrothiazole) have been found to inhibit estrus in intact mice, a study of their effects on subestrus of ovariectomized mice was deemed worthwhile.

Methods and materials. C_3H female mice of the author's colony, originally obtained from Dr. J. J. Bittner, were ovariectomized and maintained on Purina Laboratory Chow. Drugs were administered in the diet after grinding with mortar and pestle and mixing with ground food. Control and drug-diets were fed *ad libitum*, except when otherwise noted. Vaginal smears were obtained by the lavage method. Mice were housed, usually 4 to 5 in a cage, in an air-conditioned, darkened-window room with automatic light cycles of 14 hours a day.

Mice were divided into 2 groups. Group I consisted of 60 animals ovariectomized between 8½ and 9½ weeks of age and placed on various drug-diets after subestrus was well established. Group II was composed of 88 mice ovariectomized between 9 and 14 weeks of age and put on drug-diets one week after ovariectomy.

A mouse was considered to be in subestrus

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