lymphocytes in the blood by a factor of 10. General food restriction elicited a similar response in pregnant females but not in young males. The zinc deficiency regime had no effect upon the total leukocyte count or upon the concentration of zinc in the leukocytes.

Addendum. Since preparing this manuscript a recent publication by Mills, C. F., Dalgarno, A. C., Williams, R. G., and Quarterman, J., has come to our attention (Br. J. Nutr. 21, 751, 1967). These workers have reported a rapid fall in the plasma zinc levels of zinc-deficient calves and lambs similar to our own findings in rats.

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Transitory Impairment of Interferon Production in Serotonin Treated Mice* (32972)

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Interferon production appears to represent an important defense mechanism against viral infections. While studies have shown the influence of steroid hormones both on interferon production (1) and on susceptibility to infection (2), reports are limited which relate vasoactive amines to such responses (3). Based on the observations that certain amines, e.g., epinephrine, histamine, and serotonin, are secreted in response to various stimuli and that alterations in interferon production (4) and in susceptibility to viral infections (5) have also been noted in response to noxious stimuli, a study was undertaken to determine if a correlation between these observations could be demonstrated. This report describes the influence of serotonin (5-hydroxytryptamine; 5-HT) on interferon production.

Materials and Methods. Animals. Both male and female, 7 to 12-week-old Swiss-Webster BRVS mice were used. Sex and age were held constant within each experiment.

Serotonin. Mice were injected i.p. with 1-mg doses of 5-HT as the creatinine sulfate salt suspended in 0.25 ml of saline. Control mice were injected with 0.25 ml of saline.

Interferon stimulation and assay. At intervals from 30 min before to 8 hours after 5-HT injections, groups of 7 mice were injected i.v. with 0.2 ml of either a 1:5 dilution of egg allantoic fluid containing about 5.6×10^7 plaque forming units (pfu) of Newcastle disease virus (NDV), or with 100 μg of *E. coli* lipopolysaccharide endotox-

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in, or with 1 mg of statolon[1]. Blood was collected 4 hours after NDV and statolon injections and 2 hours after endotoxin injections. Sera from 2 or 3 mice were pooled. For the interferon assay, secondary mouse embryo tissue cultures in 30-ml plastic flasks were treated for 3 hours at 37°C with serum dilutions, then challenged with 75 to 100 pfu of vesicular stomatitis virus (VSV). After 1-hour viral adsorption, an overlay containing Tris buffer with 0.6% agar and 5% agamma globulin calf serum was added. Four flasks were used per dilution. After 40 hours incubation, the pfu were counted. The NDV-induced interferon was assayed at a 1:4000 dilution, statolon at 1:512 and endotoxin at 1:256. It had been predetermined that these serum dilutions reduced the pfu about 50% in control samples of the respective interferon systems. The results are expressed as the percentage reduction from noninterferon treated virus control flasks.

As a check on the reliability of the interferon assay, a reference stock of NDV-induced interferon was maintained at —20°C, and was assayed with each experiment. Four duplicate samples, 3 flasks each, at 1:4000 dilution were tested each time; the highest mean value per assay was 67% and the lowest was 40% pfu reduction. The average mean was 48% ± 8% standard deviation.

Results. A transitory impairment in interferon producing ability was seen in mice injected with NDV from about 15 min before to 1–2 hours after 5-HT injection (Fig. 1). No impairment was seen in mice injected with NDV 30 min before 5-HT injection, and by 4 hours after 5-HT injection, mice again produced normal amounts of interferon. Similar results were seen with statolon (Fig. 1). On the other hand, no impairment of endotoxin induced interferon production was seen in 5-HT treated mice (Fig. 1).

As the spleen is a prominent interferon producing organ (6) and as secretion of adrenocortical steroids can be stimulated by 5-HT (7) which may in turn reduce interferon production (1), experiments similar to

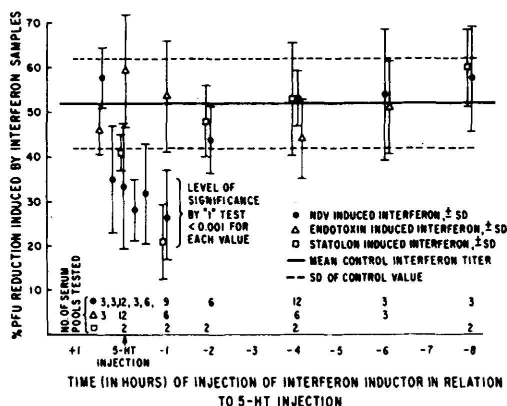

FIG. 1. Interferon levels induced in mice by NDV, endotoxin, or statolon given at intervals in relation to 1-mg injections of 5-HT. Vertical lines indicate standard deviations (SD) from mean. The average mean control value for all experiments was 52%; the mean values from individual experiments ranged from 42 to 68%. Points on the graph indicate the percentage deviation of experimental groups from their individual controls, not from the average control value.

those above using NDV were conducted with splenectomized and with adrenalectomized mice. The influence of 5-HT on interferon production was found not to be mediated by either the spleen or adrenal glands (Table I). The non-5-HT treated splenectomized mice produced less interferon, as also observed by Fruitstone, *et al.* (6), while the adrenalectomized mice produced slightly more interferon than intact mice; yet the transitory 5-HT induced impairment of interferon production was still seen in both groups.

Experiments were next conducted which showed that impaired interferon production was directly related to the action of 5-HT. Mice were treated at intervals from 15 min before to 1 hour after the 5-HT injections with a subcutaneous injection of 0.02 mg methysergide,[2] a 5-HT antagonist. The NDV was given 1 hour after the 5-HT injection. Mice given methysergide 15 min before or at the same time as 5-HT experienced no impairment of interferon production (Table II). When methysergide was given

[1] Kindly supplied by Eli Lilly Co., Indianapolis, Indiana.

[2] Kindly supplied by Sandoz Pharmaceuticals, Hanover, New Jersey.

TABLE I. Comparison of NDV Induced Interferon Production in 5-HT and Non-5-HT Treated Splenectomized and Adrenalectomized Mice.

Time of interferon induction after 5-HT injection (hours)	pfu reduction (%) from virus controls ± SD[a] in: Splenectomized mice 5-HT	Splenectomized mice No 5-HT	Adrenalectomized mice 5-HT	Adrenalectomized mice No 5-HT
0			31 ± 2	
1	14 ± 13[b]	40 ± 15	29 ± 15[b]	64 ± 13
4	41 ± 9		59 ± 8	61 ± 4
8	35 ± 6	38 ± 3		

[a] SD = standard deviation.
[b] $p < .05$, 3 serum pools per group.

beyond 15 min after 5-HT injection, no significant blocking influence was seen. This also demonstrated that the major influence of 5-HT was mediated within the first 15 min after injection.

The influence of dosage and route of 5H-T injections were also studied. Doses of 0.1 mg induced a moderate (15% below the interferon control) reduction in interferon production. Doses of 0.01 mg and lower induced no significant change. When 1 mg was injected i.v. the transitory impairment of interferon producing ability was of shorter duration, being maximum (25% reduction below the interferon control) immediately after injection and having returned to normal 1 hour later. Subcutaneous injections induced a response similar to the i.p. injections.

TABLE II. Blockage of 5-HT Induced Impairment of Interferon Production by Methysergide Given Before or at Same Time as 5-HT.[a]

Interferon was induced by NDV injected 1 hour after 5-HT.

Time of methysergide injection in relation to 5-HT injection (min)	pfu reduction (%) from virus controls ± SD
+15	55 ± 6
0	51 ± 8
−15	32 ± 8
−30	32 ± 7
−60	22 ± 9
Controls (no methysergide injection)	
5-HT	23 ±10
Saline	58 ± 8

[a] Three serum pools per group; SD = standard deviation.

TABLE III. Influence of 5-HT on the Rate of Interferon Production.

Time after NDV injection (hours)	pfu reduction[b] (% ± SD) in: 5-HT treated[a]	Controls[a]
2	3 ± 2	13 ± 3
4	26 ±12	54 ± 6
6	54 ± 7	77 ± 5
8	73 ± 5	98 ± 3
10	72 ± 8	97 ± 2
12	57 ± 6	75 ± 9
16	25 ± 9	54 ± 3

[a] 7 mice and 3 serum pools per interval.
[b] At 1:4000 serum dilutions.

To determine if the differences seen at 4 hours were due to a delay in the rate of production or in the total amount of interferon produced, serum samples were collected at 2-hour intervals for 16 hours from mice stimulated with NDV 1 hour after a 5-HT injection. Maximum amounts of interferon were found in both 5-HT treated and control mice by about 8 hours, and a reduced amount was seen consistently in the 5-HT treated mice over the 16-hour period (Table III).

The possible effect of 5-HT on the tolerant state to interferon production, that is observed up to 72 hours after a primary stimulation with virus (8), was next examined. One group (A) of mice was injected first with 5-HT followed in 30 min by the first NDV injection and in 48 hours by the

TABLE IV. Effect of 5-HT on Tolerance to Interferon Induction.

Group[a]	Pretreatment	Serum[b] dilutions ± SD inducing 55% pfu reduction[c]
A	5-HT + NDV after 0.5 hours	1:1249 ± 94
B	5-HT + NDV after 6 hours	1:533 ± 48
C	NDV only	1:630 ±119
D	None	1:4000

[a] Seven mice and 3 serum pools per group.
[b] Collected after second NDV injection.
[c] Determined by Reed-Muench formula.

second NDV injection. Group B received the first NDV injection 6 hours after and the second 48 hours after the 5-HT injection. Group C received only 2 NDV injections 48 hours apart. Group D, serving as interferon control, received only 1 NDV injection. Sera were collected 4 hours after last NDV injection. Sera from group D were assayed at a 1:4000 dilution and at dilutions ranging from 1:500 to 1:4000 for the other groups. The results are recorded as the calculated dilutions which induced the same pfu reduction (55%) as group D (Table IV). The tolerant state was less pronounced in group A, compared to groups B and C.

Various concentrations of 5-HT had no suppressing influence on virus induced interferon production in mouse peritoneal leukocytes maintained *in vitro*.

Discussion. These data indicate a direct and rapid action of 5-HT on the interferon producing abilities of intact animals. Several possible modes of action are suggested. First, the transient nature of the impaired capacity to produce interferon correlates generally with vasoconstriction which is one of the major physiological responses to 5-HT. Such a response might reduce the number of interferon producing cells which are exposed to the inducing agent. This hypothesis is supported by the ability of mice challenged initially with virus shortly after 5-HT injection to produce a greater amount of interferon upon a second viral injection during the otherwise tolerant state (Table III); thus, indicating that a certain number of cells were not stimulated to produce interferon after the initial viral injection.

The evidence also suggests a possible interference with the metabolic production of interferon, as indicated by the failure of 5-HT to impair the production of preformed nonmetabolized interferon, which is induced by endotoxin (9), while the production of newly synthesized interferon, which is induced by both NDV and statolon (10), was impaired. The failure of 5-HT to impair interferon production *in vitro* does not support this mode of action; however, caution must be used in comparing *in vitro* and *in vivo* systems.

The significance of the 5-HT impairment of interferon production on overall host–virus relationships is not known. We are currently conducting experiments to determine if alterations in susceptibility can be correlated with these transitory reductions in interferon. Lavender (11) demonstrated a marked transitory increase in susceptibility, due to possible alterations in tissue barriers, of mice simultaneously injected i.v. with neurotropic viruses and either 5-HT, epinephrine, or norepinephrine. These studies suggest that vasoactive amines may have a transitory influence on natural resistance to viral infections.

Summary. The ability of mice to produce either virus or statolon induced interferon was temporarily impaired (for 1–2 hours) following an injection of serotonin. This impairment was also noted in splenectomized and in adrenalectomized mice. Endotoxin induced interferon was not influenced by serotonin. The possible mechanisms of this impairment are discussed.

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Enhancement of Intestinal Iron Absorption by a Humoral Effect of Hypoxia in Parabiotic Rats (32973)

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In recent years much has been learned about the kinetics of the mechanism for the intestinal absorption of iron and the diverse conditions associated with increased iron absorption (1–4); yet the link between these conditions and the absorptive process is unknown. The possibility that a humoral factor might be involved has led us to use parabiotic rats in an attempt to determine if iron absorption can be promoted by an indirect effect (5,6). The results indicate that hypoxia can increase iron absorption when the possibility of a direct effect on the bowel is excluded and that this action must be mediated by a transferable humoral factor. Our findings, with those of other workers, suggest that this factor probably is not erythropoietin.

Materials and Methods. Highly inbred female rats of strain LEW/Mai, weighing 60–75 gm each, were parabiosed by a modification of the Bunster-Meyer technique. These inbred animals were selected because parabionts could be prepared without the occurrence of the intoxication reaction observed with outbred strains. Twelve of 19 pairs were successfully used in the study. Of the remaining seven pairs, three died spontaneously, two were killed accidentally, one did not gain weight normally, and one was excluded because of imperfect dosing. They were given Purina rat chow *ad libitum*, and 4–5 weeks later, when the combined weight of a pair was approximately 250 gm, hematocrits were measured in blood taken from the tail vein of each animal. Patency of the vascular anastamosis between parabionts was established by injecting a small quantity of red cells labeled with ^{51}chromium into the tail of Rat A, the member that would subsequently be subjected to hypoxia, and 3–6 hours later measuring the radioactivity in 40 μl of tail vein blood from Rat B. Equilibration of injected red cells occurred within 3 hours. The parabiotic rats continued to grow normally: they did not develop anemia, parabiotic shift of red cell mass or intoxication. Occasional testing of vascular anastamoses with ^{51}chromium labeled red cells during the course of the experiment showed them to be functional in all cases.

Intestinal iron absorption was measured as follows: both members of a pair were starved but given tap water *ad libitum* for 24 hours. Rat B was then given 0.6 ml of fluid containing 0.2 μC ^{59}ferrous citrate and 500 μg of carrier iron as freshly prepared ferrous sulfate via a polyethylene tube passed down the esophagus into the stomach. Each pair was then housed individually in a stainless steel cage which was designed to permit collection on aluminum foil of all feces passed by both rats. After 6 days the feces were put into a polyethylene container (250-ml capacity) and the aluminum foil was washed extensive-

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