that no net uptake of K+ occurs in the presence of intracellular acid but in the absence of metabolism. Furthermore, a relatively linear relationship exists between fermentation rate and the dependent K+ uptake rate. Hence the acid appears to serve solely as a tool for inducing fermentation; fermentation in turn allows K+ uptake. These findings are in direct conflict with those of Leggett *et al*(3) whose claims are listed above. It is possible that Leggett *et al* were dealing with a single strain or mutant which *does* transport K+ passively. The possibility also exists that Leggett *et al* damaged the cells by pretreatment with acid; such damage might affect K+ transport.

Finally, an interesting direct relationship between K+ uptake and metabolism has been obtained. In previous studies of K+ uptake with sugar, metabolic rate was so high and K+ uptake so fast, that it would be difficult to measure the relative rates. The present findings, by virtue of the easily measured rates of both fermentation and K+ uptake, may present an approach to discovery of direct quantitative relationships between metabolism (energy expenditure by the cell) and transport. The technique must be improved in order to do so.

Summary. 1. In acid-induced fermentation in commercial baker's yeast, no direct relationship was seen between acid penetration and K+ uptake. A dependency of K+ uptake on metabolism was seen, however. 2. Evidence is presented in support of the concept of an "active" process in the transport of K+ into yeast. This evidence conflicts with recent contentions of a "passive" process in K+ transport in yeast. 3. A direct approach may be afforded to studies of energy expenditure by the yeast cell and K+ transport.

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Stress and Susceptibility to Viral Infections. III. Antibody Response and Viral Retention During Avoidance Learning Stress.* (29342)

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Mice subjected to emotional or nonspecific stress by an avoidance-learning situation in a shuttlebox, by confinement in mesh wire envelopes, or by high intensity sound are more susceptible than controls to the viruses of herpes simplex, Coxsackie B1, and vesicular stomatitis(1,2,3).

Two to four weeks of avoidance-learning stress were necessary before significant differences were noted in susceptibility of mice to herpes simplex and Coxsackie B1 viruses given by intraperitoneal inoculation(1,2). Sound stress(3) resulted in a very transient and diphasic susceptibility pattern in mice inoculated intranasally with vesicular stomatitis virus; these changes were present as early as the first day of stress. Furthermore, the periods or phases of increased susceptibility in sound-stressed mice were not dependent on adrenal functions. Leukocyte

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counts were also biphasic, decreasing during and increasing after the stress period(4).

Physiological changes observed in stressed mice were adrenal hypertrophy after 2-3 days and splenic and thymic atrophy after 2 weeks; additional changes were minimal after 2 weeks(5).

Our present report describes the rate of viral disappearance from the site of inoculation and the immune response in intact and adrenalectomized mice subjected to avoidance-learning stress and inoculated intramuscularly with vesicular stomatitis virus.

Materials and methods. The avoidance-learning stress procedure in a shuttlebox apparatus has been described(1). Six to 8 week old female Swiss mice, Webster strain, were stressed 6 hours per day. Acutely stressed mice were those inoculated on the second day of stress, and chronically stressed were those inoculated on the 15th day. Control animals remained in home cages from which food and water were removed for 6 hours daily.

Inoculum. Vesicular stomatitis virus (VSV), New Jersey type, grown in 9 day old chick embryos, was used. Each mouse was inoculated in the inner aspect of the thigh muscle with 0.05 ml of chorioallantoic fluid diluted so as to contain 2×10^6 plaque forming units (pfu), ordinarily 1:10. Mice were inoculated just before the stress period and daily stressing was continued until day of sacrifice.

Antibody and viral assay. At intervals after inoculation, stressed and control mice were sacrificed just before the daily stress period, blood was collected for antibody titration, and muscle was collected for viral assay. Antibodies were measured by the 50% plaque reduction method(6); virus concentrations in muscle were determined by plaque assay on secondary chick embryo tissue cultures grown in plastic flasks (Falcon Plastics Co., Los Angeles).

Results. Antibody titrations. The antibody titrations in chronically stressed and control mice are shown in Fig. 1. Antibody levels of 1:4 were detectable by the second day after inoculation, and increased steadily to a titer of about 1:1024 by the fifth day after

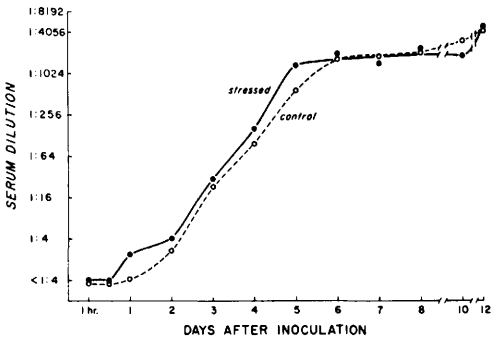

FIG. 1. Antibody response in chronically stressed and control mice, inoculated I.M. with VSV, as determined by the 50% plaque reduction method. Each point represents mean value of 12 mice from 4 separate experiments.

inoculation. No significant differences were noted between stressed and control animals. Similar results were obtained in acutely stressed mice and in stressed and non-stressed adrenalectomized mice.

Viral assays. The data presented in Table I show viral concentrations in the inoculated muscle tissues of the various stress and control groups of mice. No significant differences in viral concentrations were found in muscle tissues of acutely stressed as compared to control mice (Group A, Table I). No significant differences were seen in chronically stressed as compared to control mice during the first 12 hours after inoculation when concentrations of detectable virus increased in both stress and control animals; however, at 1 day the concentration of virus was as much as 60 times greater in the stressed mice (Group B, Table I). A decrease in virus titer was noted through the next 5 to 6 days until no further virus could be detected. Throughout this period the muscles of chronically stressed mice generally contained more virus than did control muscles.

An explanation for the above differences between chronically stressed and control mice might be impairment of host defense mechanisms secondary to increased secretion of adrenocortical steroids. To test this hypothesis mice were stressed for 2 weeks, then adrenalectomized and inoculated as above. Controls were similarly adrenalectomized and inoculated (Group C, Table I). Again, a

TABLE I. Virus Concentrations in Muscles of Stressed and Control Mice. Mean values represent pfu of VSV per 0.1 g of tissue expressed as the negative log. P values based on "t" test for significance of difference between stressed and control groups.

Group	Mice per interval		Time of collection after inoculation													
			1 hr		12 hr		1 day		2 days		3 days		4 days		5 days		6 days		7 days	
			S*	C*	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C
A	6	Mean	3.02	3.34	3.94	4.13	4.27	4.40	4.45	3.99	4.34	4.08	4.29	4.19	1.22	.46	.56	0	0	0
		± S.D.	1.18	1.38	1.26	.85	1.03	.99	.33	1.12	.47	.95	.64	.75	2.11	.80				
		P	NS		NS		NS		NS		NS		NS		NS					
B	12	Mean	3.15	2.86	3.84	3.50	4.84	3.34	4.16	2.85	3.90	3.30	3.69	3.47	3.07	1.54	1.69	0	0	0
		± S.D.	.65	.93	.98	.68	.71	.53	.76	.81	.80	.51	.78	.78	.86	1.35	1.40			
		P	NS		NS		.001		.01		.01		NS		.01					
C	8	Mean	3.37	3.20	3.81	3.59	3.42	1.83	3.21	1.96	2.46	2.85	2.39	2.32	1.32	1.13	.57	0	0	0
		± S.D.	.41	.43	.49	.62	1.17	1.43	.78	1.32	1.32	.40	1.43	.70	1.09	1.32	1.28			
		P	NS		NS		.02		.02		NS		NS		NS					
D	6	Mean	3.54	3.17	4.60	4.53	4.82	2.00	3.31	2.57	2.31	1.09	2.11	1.46	.27	0	0	0	0	0
		± S.D.	.88	1.73	1.91	1.99	1.23		.52	.46	1.41	1.15	1.30	.87	.62					
		P	NS		NS		.01		.02		.1		NS		NS					

Group A: Acutely stressed. Group B: Chronically stressed. Group C: Chronically stressed and adrenolectomized after 2 weeks of stress. Group D: Chronically stressed and adrenalectomized before initiation of stress.
* S = Stressed, C = Controls, NS = Not significant.

greater retention of virus in the muscle of the stressed mice is noted at 1 and 2 days. These data indicate that differences in amounts of virus were not related directly to stress induced increases in adrenal secretions. To determine whether or not the stressed mice had been predisposed to greater susceptibility during the 2-week stress period by adrenal-mediated responses prior to adrenalectomy, mice were adrenalectomized before initiation of stress, stressed for 2 weeks and then inoculated (Group D, Table I). Controls were similarly tested. The results are analogous to those obtained for Group C, *i.e.*, significant differences were seen on days 1 and 2. These results further indicate that stress induced responses causing virus retention are not mediated by adrenal glands.

To determine whether or not the transitory increase in titratable virus observed during the first 12 to 24 hours after inoculation was due to viral multiplication or release from inhibition by possible antiviral substances in the inoculum, *e.g.*, interferon, virus was washed free of chorioallantoic fluids by ultracentrifugation and then inoculated into mice as above. The following virus titers expressed as the negative log were measured in the muscles: at 1 hour, 4.07 pfu; at 12 hours, 3.84 pfu; at 1 through 4 days, 3.45, 2.43, 2.30 and 0.56 pfu respectively. The early transitory increase in virus titer was not seen, thus suggesting the presence in the inoculum, which contained 10% chorioallantoic fluid, of some antiviral principle that diminished in activity over the first 24 hours after inoculation.

Discussion. Our results show no significant differences in the immune response between stressed and nonstressed animals as measured by neutralizing antibodies in serum. Similar results have been reported in cold stressed mice(7). On the other hand, in earlier studies (8) using avoidance learning stress, a suppression of the immune reaction responsible for skin homograft rejection was demonstrated in stressed mice. Suppression of delayed type hypersensitivity in stressed rats has also been shown(9). The possible role of delayed hypersensitivity in the changes in

resistance to infection observed in stressed mice deserves further study.

The increased retention of virus in the tissue of stressed mice suggests impairment of host defense mechanisms for destruction of virus. Teodoru(10) found retention of polio virus in interscapular brown fat of hamsters subjected to stress and/or cortisone injections, and suggests that the stress-response is a reflection of hypercortisonemia initiated by stress. Our results also show a greater retention of virus in the tissues of stressed animals, but the experiments using adrenalectomized mice, in which similar retention was found, indicate no correlation between adrenal secretions and this retention. The generally held concept that increased adrenal cortical secretions during stress universally explain the diminished resistance to infectious diseases in stressed animals is not supported by our present data nor by data from our earlier studies with sound stress(3).

Summary. Mice subjected to avoidance-learning stress for 2 weeks were inoculated intramuscularly with vesicular stomatitis virus. By measuring serum neutralizing antibodies, no differences in immune response between stressed and control mice were noted. Virus disappearance from the site of inoculation was significantly retarded in stressed mice. Analogous results were obtained with adrenalectomized mice.

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An *in vitro* Effect of Insulin on the Preputial Gland.* (29343)

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Although insulin has been shown to act on the carbohydrate metabolism of a variety of tissues *in vivo* and *in vitro,* documentation of its effects on skin have been sparse. O'Connor demonstrated temperature conditioned changes in oxygen consumption of mouse tail skin following *in vivo* administration of insulin(1). Gaylor documented enhanced incorporation of acetate-C^{14} into squalene in isolated rat skin incubated with insulin and glucose in an atmosphere of nitrogen(2). Adachi reported increased glycogen C^{14} in the dog skin flap perfused with glucose-U-C^{14} and insulin(3).

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While the physiological relevance of the first two observations remains doubtful, that insulin effects glucose metabolism in whole skin seems probable from the findings in the dog skin. However, in all these reports insulin effects have been examined in whole skin and no distinction drawn between effects on its two distinct components, *i.e.,* the dermis and the epidermis with its appendages. This distinction assumes some importance since the clinical manifestations of insulin lack (*i.e.,* diabetes) appear largely confined to alterations in the collagen and elastic tissue as well as the vasculature of the dermis, *e.g.,* necrobiosis lipoidica diabeticorum, poor wound healing and other less obvious changes