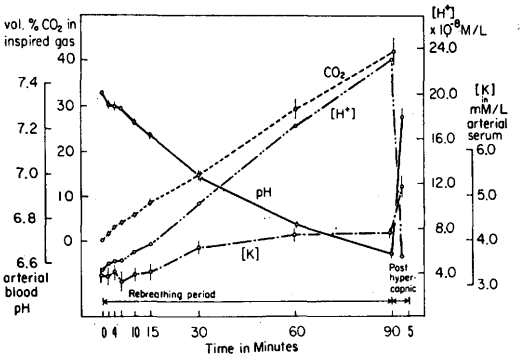

FIG. 1. Changes in plasma potassium concentration and blood pH during and following 90 min of increasing CO_2 concentration produced by rebreathing. Points are mean values of 10 experiments; vertical bars represent standard error on either side of mean.

ues within 5 minutes. During this time, plasma potassium rose abruptly to the highest values observed during these experiments. This increase, which averaged 1.04 meq/l, was highly significant.

Discussion. The transient rise which occurs in plasma potassium concentration in cats breathing high concentrations of carbon dioxide has been attributed to a stimulation of the sympatho-adrenal system resulting in a release of potassium from the liver in response to elevated circulating adrenalin(6). It has been demonstrated that this transient rise can be eliminated by sympathetic blocking agents or by removal of the liver. It seemed unlikely that this explanation would account for a transient rise in dogs that were exposed to a gradual increase in CO_2 tension from rebreathing in a bag since less stimulation of the sympatho-adrenal system would be expected under these circumstances than with sudden exposure to 30% CO_2(5). However, since it has been reported that this transient rise occurred, this phenomenon was investigated.

In our experiments no evidence of a transient rise in plasma potassium was found in any case. No satisfactory explanation is available to account for the difference in results in the experiments of Adrian and Gordon and those reported here. The physicochemical basis of the exchange of potassium between intracellular and extracellular fluids has also been investigated(7).

Summary. Plasma potassium was determined on dogs before and during 90 minutes of rebreathing, and immediately following return to air breathing. Potassium concentration rose gradually with increasing CO_2 tensions during the first 60 minutes of rebreathing and remained essentially constant for the last 30 minutes. On changing from the high CO_2 concentration to air breathing, a further rapid rise in plasma potassium concentration appeared. No evidence of a transient early rise in potassium concentration was observed.

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Effects of Octacosanol on Chick Comb Growth. (28034)

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Previous studies from this laboratory(1) indicate that wheat germ oil contains an unidentified factor or factors which when assayed by the method of Dorfman and Dorfman(2) resulted in a significant increment in chick comb growth. Subsequent studies*

* Ezra Levin, unpublished findings.

TABLE I. Effects of Natural and Synthetic Octacosanol on Chick Comb Growth (22 Animals/Group).

Material inuncted	Total dose, μg/0.20 cc	Avg ratio*	Diff. from control	Variance	"t" value
Cottonseed oil† control		27.43		.50	
Cottonseed oil† + following:					
Natural octacosanol	1296	31.34	3.91	1.25	2.94
" "	3.6	30.57	3.14	.78	2.78
Synthetic octacosanol	1296	32.52	5.09	1.05	4.07
" "	216	32.71	5.28	1.73	3.52
" "	27	31.86	4.43	.95	3.66
" "	3.6	30.75	3.32	.73	2.99
" "	.36	34.62	7.19	1.75	4.79
" "	.036	36.10	8.67	.60	8.26
" "	.0036	32.40	4.97	2.51	2.86
" "	.00036	31.70	4.23	.59	4.03
Testosterone propionate	4.0	31.51	4.08	1.76	2.72

* Ratio $= \frac{\text{Comb wt in mg}}{\text{Body wt in g}} \times 100$.

† Egg lecithin was added to and thoroughly mixed with cottonseed oil (Wesson, Wesson Oil & Snowdrift Sales Co., New Orleans, La.) in the ratio of 8 parts egg lecithin to 92 parts cottonseed oil.

demonstrated that octacosanol, a 28 carbon atom, straight chain, saturated alcohol derived from wheat germ oil, had similar activity. The following investigation was undertaken to confirm and extend these earlier findings.

Procedure and results. Assays were conducted by the procedure of Dorfman and Dorfman(2). In Experiment 1, two hundred and sixty-four male White Leghorn chicks† were selected at one day of age and divided into 12 comparable groups of 22 birds each. Animals were placed in thermostatically controlled brooders and were provided with chick starting mash‡ and water *ad libitum*. Data were obtained on the comparative effects of graded levels of synthetic octacosanol, 2 levels of "natural" octacosanol,§ derived from wheat germ oil, and a daily dose of 1 μg of testosterone propionate on chick comb growth. The total dose of test material was contained in 0.20 cc of cottonseed oil; and 0.05 cc of the test solution was applied daily for 4 consecutive days on the comb of each chick (starting at 1 day of age). Twenty-four hours after the last application, the animals were sacrificed and body weight and comb weights were determined. Comb responses were expressed as the ratio of chick comb to body weight. The average body weight of chicks at start of the assay was 39.9 g and at time of sacrifice, 53.8 g. Differences in weight increment between the various groups were not statistically significant. Both synthetic octacosanol and octacosanol isolated from wheat germ oil caused a highly significant increment in chick comb growth.‖ Essentially the same quantitative response occurred over a dosage range from 0.00036 μg to 1296 μg. The increment in chick comb growth induced by a dose of 0.00036 μg octacosanol was comparable to that induced by over 11,000 times this amount (*i.e.*, 4 μg) of testosterone propionate. Findings are summarized in Table I.

In Experiment 2 data were obtained on the comparative effects of octacosanol, wheat germ oil and testosterone propionate on ratio of chick comb to body weight in 2 strains of chicks: (a) White Leghorn chicks similar

† Honegger Hatcheries, Fairbury, Ill.

‡ Corn Belt Chick Starter Mash, Corn Belt Mills, Inc., Gibson City, Ill.

§ Isolated by procedure described in U. S. Patent No. 3,031,376, April 24, 1962.

‖ Subsequent studies demonstrated that octacosanol is not the only straight chain saturated alcohol with the activity indicated above. Similar activity was exhibited by a number of compounds with the formula: R-O-X where R is a straight chain alkyl radical having 24, 26, 28, 30, 32 or 34 carbon atoms and X is either H or an acid radical.

TABLE II. Comparative Effects of Octacosanol, Wheat Germ Oil and Testosterone Propionate on Chick Comb Growth in Birds of White Leghorn and Hy-Line Strains (30 Animals/Group).

Material inuncted	Total dose, μg/0.25 cc	Avg ratio*	Diff from control	Variance	"t" value
		White Leghorn strain			
Cottonseed oil† control		26.57		.47	
Cottonseed oil† + following:					
Synthetic octacosanol	4.5	32.89	6.32	.85	5.50
" "	.045	32.39	5.82	1.37	4.43
" "	.0045	33.67	7.10	1.09	5.68
Wheat germ oil—1:60		31.92	5.35	1.45	3.85
Testosterone propionate	5.0	32.38	5.81	1.20	4.47
		Hy-Line			
Cottonseed oil control		32.35		1.56	
Cottonseed oil + following:					
Synthetic octacosanol	4.5	33.08	.73	1.66	.41
" "	.045	32.34	− .01	1.12	negative
" "	.0045	29.80	−2.55	1.47	"
Wheat germ oil—1:60		30.85	−1.50	.69	"
Testosterone propionate	5.0	33.96	1.61	1.57	.91

* † See footnotes, Table I.

to those employed in Exp. 1 and (b) a hybrid strain.¶ One hundred and eighty male chicks of each of the above strains were divided at one day of age into 6 comparable groups of 30 animals each. These were treated with the materials indicated in Table II. The experimental procedure was similar to that employed in Exp. 1 except that the test solutions were applied daily for 5 consecutive days instead of 4. Animals were sacrificed 24 hours after the last application and ratio of chick comb to body weight determined.** A significant difference in response was observed between the White Leghorn and hybrid strains. In the White Leghorn strain in agreement with findings in Exp. 1, both testosterone propionate and octacosanol at the levels tested resulted in comparable and highly significant increments in the ratio of chick comb to body weight. Wheat germ oil when mixed with cottonseed oil in the ratio of 1 part wheat germ oil to 59 parts cottonseed oil had a similar effect. With the hybrid strain, however, none of the substances tested caused a significant increment in ratio of chick comb to body weight.

No data are available as to the mechanism whereby octacosanol exerts its stimulatory effect. Inasmuch as a dose-response effect was not obtained with this material in contrast to that shown by graded doses of testosterone propionate(2), it would appear that the stimulation of chick comb growth with octacosanol in birds of the White Leghorn strain was not a true androgenic effect. The failure of octacosanol to stimulate chick comb growth in birds of the hybrid strain suggests that the physiologic activity of this material is influenced by the genetic makeup of the animal tested.††

Summary. Topical application of octacosanol, a 28 carbon atom, straight chain, saturated alcohol, on the combs of immature White Leghorn chicks caused a highly significant increment in chick comb growth. In contrast to results with testosterone propionate, a dose-response relationship was not obtained. A strain difference in response to octacosanol administration was observed.

¶ Hy-Line chicks, supplied by Pioneer Hi-Bred Corn Co., Topeka, Kan.

** These studies were conducted in collaboration with Dr. G. B. Marion, Dept. of Dairy Husbandry, Kansas State Univ., Manhattan, Kan. The assistance and cooperation of Dr. Marion is gratefully acknowledged.

†† The lack of effect of octacosanol and testosterone propionate in stimulating chick comb growth in Hy-Line chicks is in keeping with previous reports of strain differences in response(3).

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Effect of Cold and Restraint on Intracellular Distribution of Glutathione in Rat Liver.* (28035)

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Beck and Linkenheimer(1) first showed that shock and cold stress decreased the nonprotein sulfhydryl (NPSH) levels in mouse liver. Bartlett and Register(2,3) reported that cold or restraint alone markedly decreased rat liver NPSH. This decrease in NPSH was shown to be primarily glutathione (GSH)(4).

The present study was undertaken to determine which intracellular fraction of the rat liver exhibited changes in GSH during stress of cold and restraint.

Methods. Female, Sprague-Dawley rats, weighing between 200 and 250 g, were employed for this experiment. These rats were previously kept on a stock diet. One-half of these animals was sacrificed and used as controls. The other half was stressed as previously described(3,5) by putting the animals in loosely fitting cages and placing in a cold room kept at 0° ± 2°C. Body temperature was so controlled that, as recorded rectally, it dropped from normal to 19-20°C over a 4 hour period. The animals were then sacrificed by decapitation and the livers were perfused with cold 0.9% saline.

The excised tissue was homogenized in 0.44 M sucrose, centrifuged at 600 *g* for 10 minutes to sediment the nuclei and cellular debris. The decanted homogenate was spun at 24,000 *g* for 15 minutes to sediment the mitochondria. The mitochondrial fraction was washed once in 0.44 M sucrose. The supernatant from the mitochondrial preparation was designated the soluble fraction. NPSH, GSH, and nitrogen was determined on the cellular fractions. NPSH was assayed by amperometric titration(8,9,4), GSH by the alloxan "305" method(4,10), and nitrogen by a standard Kjeldahl procedure.

Preliminary studies on intracellular distribution of rat liver GSH revealed that the nuclei, as prepared by the method of Chauveau *et al.*(6), or that of Hogeboom and coworkers(7), and the microsomal fraction made no significant contribution to total liver GSH. Therefore, the nuclei were removed with the cellular debris before the homogenate was used and the microsomes were not removed from the soluble fraction.

Results and discussion. Stress of cold and restraint results in a marked decrease in liver NPSH, which is principally GSH (Table I). A comparable decrease occurs in the soluble fraction with no significant change in the mitochondrial GSH. Thus the entire loss is from the soluble fraction. In stress the relative contribution of mitochondrial GSH to total liver GSH increases from 4% to 18%.

Barford and Eden(11) reported a similar intracellular distribution in rat liver with the exception of the nuclear fraction. They reported that the nuclear fraction contributed about 6% of total cellular GSH. In the present study using 2 later methods of isolating cell nuclei it was found that the nuclei contributed less than 1% of cellular GSH. No satisfactory explanation can be given for this difference.

This decrease of GSH in the soluble fraction with no change in mitochondrial fraction during cold stress was similar to the results

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