concentrations of CPDS studied, less than 10 per cent of the 6MNA liberated is due to formation of mixed disulfides of type B.

Manometric experiments, carried out with the conventional Warburg technique,[5] showed that treatment of EA cells with 10^{-3} M CPDS, followed by washing, does not alter their rates of respiration and glycolysis. Respiration and glycolysis of EA cells in the presence of 10^{-3} M CPDS are also essentially unaffected. The results of preliminary experiments indicating that CPDS is not toxic are consistent with these findings.

The results reported in this paper provide a method for the determination of external (membrane-bound) and intercellular SH groups. The possibility is opened, through the reactions described here, of binding selectively the external SH groups of EA cells, thus preventing their normal reactions.

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Arequipa Foundation,
Russ Building,
San Francisco, Calif. 94104, U.S.A.

D. R. GRASSETTI
J. F. MURRAY, JR.

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Effect of 5-methylpyrazole-3-carboxylic acid on plasma free fatty acids and blood sugar in geese*

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THE ADIPOKINETIC effect of the catecholamines can be inhibited by a number of compounds,[1] but there is less information regarding the influence of pharmacological agents on the adipokinetic effect of glucagon. Because the lipolytic effects of catecholamines and glucagon are believed to be mediated by the same biochemical mechanism,[2] it is of interest to study whether the adipokinetic effect of glucagon is inhibited by substances known to inhibit the adipokinetic effect of the catecholamines.

The pyrazole derivative, 5-methylpyrazole-3-carboxylic acid (U-19425), has been shown to decrease the plasma free fatty acid (FFA) concentration of eviscerated rats and of intact rats receiving subcutaneous injection of glucose, and to inhibit the release of FFA from rat adipose tissue *in vitro*.[3] This compound lowers plasma FFA and blocks the adipokinetic effects of epinephrine and of human growth hormone in man.[3, 4]

The present report describes the results of experiments designed to test the effect of 5-methylpyrazole-3-carboxylic acid on the elevations of plasma FFA and blood sugar produced by glucagon in geese.[5]

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Ten adult, male, domestic geese (Embden) kept in an air-conditioned room (22°) lighted for 10 hr every day were used. A commercial diet* was fed *ad lib.*, except for the day prior to the experiment when food was removed from the cage 16–18 hr before the first blood sample was taken. Experiments were done using two geese at a time. One of them received three injections each of 4·0 mg/kg of 5-methylpyrazole-3-carboxylic acid (i.m.) given, respectively, at 120, 60 and 5 min before the administration of 20·0 μg/kg of glycagon (i.v.). The other goose received glucagon alone. After completing the injection of the 10 geese, 4 weeks were allowed and the experiments were repeated, reversing the treatments. The methylpyrazole† was injected intramuscularly dissolved in saline, after adjusting the pH to 7·6 with 1 N NaOH. Crystalline glucagon‡ was dissolved in 0·1 M glycine buffer (pH 9·5) and injected into a wing vein.

Blood samples were taken from a wing vein at 120, 60 and 5 min, just before the glucagon injection, and at 5, 15, 30, 45, 60, 90 and 120 min thereafter. The blood samples taken 120 and 60 min before the injection of glucagon were obtained immediately before the corresponding injections of 5-methylpyrazole-3-carboxylic acid. The blood was collected into Corex centrifuge tubes containing NaF and kept in an ice-water bath. After removing an aliquot for blood sugar determination, the blood was centrifuged in a refrigerated centrifuge (−2°) and the plasma was separated for the determination of FFA. Blood sugar was estimated by Nelson's modification of the Somogyi method.[6] Plasma FFA were measured by the method of Trout *et al.*[7] as modified by Davis.[8]

The statistical significance of FFA and blood sugar differences was evaluated by the *t*-test for paired variates using Crow's chart.[9]

TABLE 1. PLASMA FFA AND BLOOD SUGAR OF GEESE INJECTED WITH 20·0 μg/kg OF GLUCAGON (i.v.) WITH AND WITHOUT PREVIOUS ADMINISTRATION OF 5-METHYLPYRAZOLE-3-CARBOXYLIC ACID*

Treatment	Time (min) −120	−60	0†	5	15	30	45	60	90	120
	Free fatty acids (mequiv./l.)									
Methylpyrazole‡	0·86 ±0·20	0·92 ±0·20	0·91 ±0·27	2·58 ±0·46	2·45 ±0·50	2·02 ±0·37	1·77 ±0·31	1·58 ±0·35	1·40 ±0·22	1·16 ±0·28
No methylpyrazole	0·99 ±0·22	1·06 ±0·25	0·96 ±0·17	2·46 ±0·54	2·45 ±0·41	1·99 ±0·41	1·89 ±0·41	1·71 ±0·45	1·52 ±0·36	1·25 ±0·26
	Blood sugar (mg/100 ml)									
Methylpyrazole‡	125 ±37·8	137 ±45·3	133 ±42·9	169 ±60·9	225 ±88·2	240 ±88·7	214 ±79·7	169 ±67·4	123 ±46·1	120 ±24·7
No methylpyrazole	120 ±35·7	123 ±22·9	134 ±48·9	155 ±51·7	217 ±61·9	221 ±75·8	202 ±55·8	149 ±41·4	129 ±36·7	136 ±51·2

* The values given are means ± S.D. for ten geese.
† Glucagon injected at zero time, immediately after taking blood sample.
‡ 5-Methylpyrazole-3-carboxylic acid (4·0 mg/kg, i.m.) was injected at 120, 60 and 5 min before the glucagon injection.

The mean FFA and blood sugar levels before and after glucagon, with and without previous injection of 5-methylpyrazole-3-carboxylic acid, are presented in Table 1.

No change of plasma FFA or blood sugar was observed in the samples taken 60 min before and just before glucagon injection, when the pyrazole derivative was injected. Statistical analysis showed no significant difference between the FFA and blood sugar responses to glucagon given alone or after the injection of 5-methylpyrazole-3-carboxylic acid.

* Purina Duck Growena, Ralston Purina Co., St. Louis, Mo.
† 5-Methylpyrazole-3-carboxylic acid (U-19425), lot 7465-ELF-146.17, kindly donated by the Upjohn Company, Kalamazoo, Mich.
‡ Crystalline beef-pork glucagon, lot 258, 234 B-167-a, Elli Lilly & Co., Indianapolis, Ind., kindly supplied by Drs. W. W. Bromer and W. N. Shaw.

A similar experiment was conducted in four geese given 5-methylpyrazole-3-carboxylic acid (6·0 mg/kg) by the oral route 90 min before the injection of glucagon (20·0 μg/kg, i.v.). No differences in the FFA or blood sugar responses to glucagon were noted when the results were compared with those obtained in the same animals after injection of glucagon alone and after injection of 5-methylpyrazole-3-carboxylic acid and glucagon.

As shown by the results, administration of 5-methylpyrazole-3-carboxylic acid to fasting geese had no effect on their plasma FFA and blood sugar levels. This is in contrast to the observations in rat and man reported in the literature[3, 4] and obtained with doses of 5-methylpyrazole-3-carboxylic acid smaller than those used in the present experiments.

It was also shown by our experiments that administration of 5-methylpyrazole-3-carboxylic acid had no effect on the changes of plasma FFA and blood sugar caused by injection of 20·0 μg/kg of glucagon. The effect of this dose of glucagon on the plasma FFA of geese was approximately 67 per cent of that obtained with the dose of 100 μg/kg, which was the highest dose tested in this laboratory.[5] Because nicotinic acid prevents the mobilization of FFA produced by catecholamines[10, 11] and growth hormone,[12] Hallobaugh *et al.*[4] state: "it would seem likely that the mechanism of action of the pyrazole derivative is similar to that of nicotinic acid". It is therefore of interest to note that nicotinic acid, like 5-methylpyrazole-3-carboxylic acid, is ineffective in preventing the elevation of plasma FFA produced by glucagon in geese.[13, 14] Furthermore, insulin, which antagonizes the lipolytic effects of catecholamines, does not prevent the increase of plasma FFA produced by glucagon in birds.[15] Available information indicates that glucagon is more effective in raising FFA concentration in birds than in mammals,[16, 17] whereas epinephrine is very effective in raising plasma FFA concentration in mammals, but not in birds.[18, 19]

Administration of 5-methylpyrazole-3-carboxylic acid had no effect on the hyperglycemic response to glucagon in geese, but inhibited the hyperglycemic effect of epinephrine in the rat.[3] However, 5-methylpyrazole-3-carboxylic acid does not inhibit the hyperglycemic effect of epinephrine in man.

Jay Phillips Research Laboratory,
Mount Sinai Hospital, and
Laboratory of Physiological Hygiene,
University of Minnesota,
Minneapolis, Minn. 55404, U.S.A.

RAFAEL CARMENA
FRANCISCO GRANDE

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