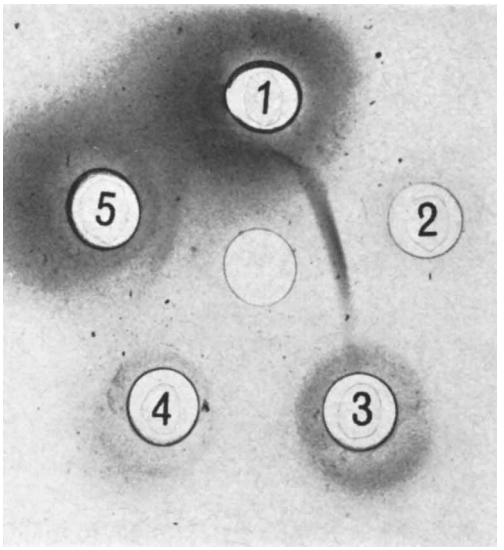

FIG. 2. Gel diffusion plate showing reaction between goat antihuman β1C/β1A (center well) and normal human serum (well 2) and serum from a rat bearing HEp 2 (well 1) and a rat bearing J-111 (well 3), but no reaction with serum from normal control rat (wells 4 and 5).

J-111 (well 5) and with normal human serum (well 2). The continuity of this precipitin line indicates antigenic identity amongst proteins in each of the three sera. There is no reaction with serum from two control rats (wells 3 and 4).

These results indicate that serum from rats bearing transplants of either of these two human cell lines contains human β1C and/or β1A serum globulins, or proteins with identical antigenic determinants. It is presumed that these proteins were produced by the human cells growing in the rats.

Summary. Human serum globulin (β1C and/or β1A) was demonstrated by the immunodiffusion technique in sera of rats bearing heterotransplants of human cancer cell lines HEp 2 or J-111, but not in serum of normal rats. Previous investigators have demonstrated that these cell lines produce β1C globulin in tissue culture.

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Effect of Glucagon on Plasma Free Fatty Acids and Blood Sugar in Birds.* (33059)

FRANCISCO GRANDE

Jay Phillips Research Laboratory, Mount Sinai Hospital and Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis, Minnesota 55455

Glucagon stimulates *in vitro* the release of free fatty acids (FFA) by the adipose tissue of the domestic fowl (1) the sparrow, and the pigeon (2,3). Carlson *et al.* (1) have reported that, in contrast with mammalian adipose tissue, the adipose tissue of the domestic fowl does not respond with increased liberation of FFA or glycerol when treated *in vitro* with epinephrine, norepinephrine, or other adipokinetic substances. Infusion of norepinephrine caused no change of FFA plasma concentration in the fowl (1).

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TABLE I. Plasma FFA in Geese Before and After Glucagon Injection.

Glucagon dose (μg/kg)	No. of animals	Plasma FFA (meq/liter, means ± SD)							
	Min:[a]	0	5	15	30	45	60	90	120
100	5	1.13 ±0.28	3.50[b] ±0.48	3.93[b] ±1.08	3.36[b] ±0.88	2.90[b] ±0.62	2.50[b] ±0.63	2.18[b] ±0.61	1.95[b] ±0.59
50	6	0.99 ±0.38	2.81[b] ±0.65	3.00[b] ±0.65	2.47[b] ±0.44	2.22[b] ±0.41	2.08[b] ±0.43	1.94[b] ±0.44	1.79[b] ±0.33
20	5	1.03 ±0.38	3.26[b] ±0.50	3.09[b] ±0.38	2.36[b] ±0.34	2.12[b] ±0.27	2.01[b] ±0.29	1.67[b] ±0.22	1.33 ±0.28
10	5	0.90 ±0.26	2.56[b] ±0.22	2.41[b] ±0.46	2.07[b] ±0.21	2.00[b] ±0.50	1.66 ±0.24	1.48 ±0.19	1.22 ±0.26
5	5	0.94 ±0.40	2.63[b] ±0.70	2.11[b] ±0.50	1.81[b] ±0.47	1.69[b] ±0.56	1.48 ±0.53	1.30 ±0.51	1.22 ±0.52
2	7	0.71 ±0.32	1.99[b] ±0.31	1.73[b] ±0.31	1.48[b] ±0.24	1.32[b] ±0.25	1.13 ±0.11	0.94 ±0.14	0.89 ±0.20
1	5	0.86 ±0.40	1.61[b] ±0.49	1.46[b] ±0.56	1.30[b] ±0.52	1.23 ±0.62	1.12 ±0.50	0.95 ±0.41	0.83 ±0.31
0.5	10	1.22 ±0.15	1.48[b] ±0.22	1.19 ±0.20	1.18 ±0.13	—	1.18 ±0.19	—	—
0.1	14	1.18 ±0.27	1.25[c] ±0.27	1.16 ±0.26	1.12 ±0.25	—	1.12 ±0.25	—	—
None (control)	9	1.03 ±0.30	1.09[d] ±0.32	0.94 ±0.22	0.93 ±0.21	0.96 ±0.20	0.95 ±0.16	0.98 ±0.17	0.93 ±0.14

[a] Time after injection in the wing vein.
[b] Significantly different from preinjection value, $p<0.01$ (t test for paired variates).
[c] Significant ($p = 0.03$).
[d] Not significant ($p = 0.35$).

These observations suggest the possibility that glucagon may play a role in the mechanism of fat mobilization in some avian species, but with the exception of the reports by Heald *et al.* (4) and Hoak *et al.* (5) there is no information as to the effect of glucagon on the plasma FFA level of birds.

Present study was on the effects of glucagon on plasma FFA and blood sugar in geese, ducks, turkeys, and roosters.

Methods. Adult male domestic geese (Embden), ducks (Peking), white turkeys, and Leghorn roosters were used. The animals were housed in an air-conditioned room (22-23°C) lighted for 10 hours every day. A commercial diet (Purina duck Growena, Ralston Purina Co., St. Louis, Missouri) was fed *ad libitum* except for the day prior to the experiments, when food was removed from the cage 16–18 hours before the injection. Crystalline glucagon[1] dissolved in 0.1 *M* glycine buffer, pH 9.5, was injected into a wing vein. Control animals were injected with equal volumes of glycine buffer.

Blood samples were taken immediately before the injection and thereafter at various intervals. The blood was collected in plastic centrifuge tubes containing heparin powder and kept in an ice-water bath. After measuring an aliquot for blood sugar determination, the blood was centrifuged in a refrigerated centrifuge (—2°C) and the plasma was separated for the determination of the free fatty acids. Blood sugar was estimated by Nelson's modification of Somogyi's method

[1] Crystalline Glucagon 258-234 B-167-1, Eli Lilly and Co., Indianapolis, Indiana, kindly supplied by Drs. W. W. Bromer and W. N. Shaw.

TABLE II. Blood Sugar in Geese Before and After Glucagon Injection.

Glucagon dose (μg/kg)	No. of animals	Blood sugar (mg/100 ml, means ± SD)							
	Min:[a]	0	5	15	30	45	60	90	120
100	5	109 ±17.8	135[b] ±7.6	163[b] ±13.8	184[b] ±21.0	204[b] ±34.2	184[c] ±36.0	174[c] ±36.2	153 ±32.8
50	6	118 ±15.8	140[b] ±12.0	180[b] ±8.0	223[b] ±13.2	234[b] ±26.9	222[b] ±35.6	174 ±60.9	137 ±59.6
20	5	110 ±16.9	130[b] ±15.5	165[b] ±19.4	191[b] ±23.4	177[b] ±16.7	156[c] ±16.7	115 ±12.4	118 ±13.7
10	5	134 ±18.9	155[b] ±26.7	193[b] ±38.8	215[b] ±44.2	197[b] ±46.7	176[c] ±33.6	145 ±19.0	145 ±18.9
5	5	124 ±15.9	158[b] ±21.2	188[b] ±22.2	174[c] ±43.0	155 ±42.0	147 ±11.9	131 ±24.2	133 ±14.0
2	7	122 ±16.3	150[b] ±22.0	175[b] ±30.0	146 ±44.8	140 ±45.1	141 ±28.3	135 ±16.3	138 ±24.2
1	5	135 ±12.4	159[b] ±21.7	181[b] ±35.2	164 ±40.0	154 ±39.4	158 ±32.6	155 ±31.6	148 ±22.4
0.5	10	126 ±11.4	143[b] ±10.7	147[b] ±5.5	134 ±15.3	—	131 ±10.3	—	—
0.1	14	121 ±11.2	126[b] ±12.2	124 ±12.9	124 ±12.2	—	126 ±11.8	—	—
None (control)	9	126 ±30.2	128 ±28.7	132 ±26.0	133 ±27.5	132 ±29.0	136 ±30.4	135 ±30.7	136 ±29.0

[a] Time after injection in the wing vein.

[b] Significantly different from preinjection value, $p<0.01$ (t test for paired variates).

[c] Significant ($p>0.01$, <0.05).

(6). Plasma free fatty acids were measured by the method of Trout *et al.* (7) as modified by Davis (8).

Results. Injection of glucagon in geese caused a prompt elevation of plasma FFA concentration as shown in Table I.

For the doses between 1 and 100 μg/kg the effect of glucagon measured by the area

TABLE III. Plasma Free Fatty Acids and Blood Sugar in Peking Ducks Before and After Glucagon Injection.[b]

	Time after injection in the wing vein (min)				
	0	5	15	30	60
Free fatty acids (meq/liter)					
Glucagon	1.29 ± 0.39	3.92[a] ± 0.80	3.63[a] ± 0.68	3.16[a] ± 0.69	2.47[a] ± 0.52
Control	0.92 ± 0.22	0.94 ± 0.24	1.00 ± 0.27	1.07 ± 0.21	1.02 ± 0.27
Blood sugar (mg/100 ml)					
Glucagon	116 ± 4.6	147[a] ± 11.0	201[a] ± 23.5	225[a] ± 24.9	184[a] ± 55.2
Control	121 ± 15.2	120 ± 6.6	124 ± 4.8	130 ± 8.3	131 ± 7.6

[a] Significantly different from preinjection value, $p<0.01$ (t test for paired variates).

[b] Means and standard deviations for 10 ducks injected with 100 μg of glucagon (mean weight 1.58 kg) and for 6 control ducks injected with glycine buffer (mean weight 1.46 kg).

TABLE IV. Effect of Glucagon Injection (10.0 μg/kg) in Turkeys.[a]

			Time after injection (min)			
	C 1[b]	C 2[c]	5	15	30	60
Free fatty acids (meq/liter)	0.42 ± 0.10	0.44 ± 0.09	1.50 ± 0.20	1.58 ± 0.15	1.05 ± 0.19	0.65 ± 0.06
Elevation above mean control value		Mean	1.07	1.15	0.62	0.22
		SE	±0.08	±0.08	±0.11	±0.04
		p	<0.0001	<0.0001	=0.002	=0.002
Blood sugar (mg/100 ml)	215 ± 24.4	216 ± 29.3	239 ± 21.3	261 ± 28.8	246 ± 24.4	222 ± 17.5
Elevation above mean control value		Mean	23	45	30	6
		SE	3.3	7.2	9.7	10.3
		p	=0.001	=0.0016	=0.03	=0.6

[a] Means and SD for 6 birds.
[b] Control sample taken 30 min before injection.
[c] Control sample taken just before injection.

under the curve of FFA concentration above the preinjection level showed a high correlation with the logarithm of the dose in μg/kg ($r = 0.981$, $p<0.01$). The relationship between dose (x) and effect (y) is described by the regression equation

$$y = 78.44 \log x + 28.67.$$

Glucagon caused an elevation of blood sugar as shown in Table II. The change of blood sugar concentration as percent of the preinjection value was smaller than the corresponding change of plasma FFA. The effects of glucagon in ducks and turkeys are summarized in Tables III and IV and the effects in two roosters are presented in Fig. 1. Like the goose these three species responded to the injection of glucagon with marked elevations of plasma FFA and blood sugar.

Discussion. The increase in plasma FFA produced by glucagon injection indicates that the adipokinetic effect of glucagon observed in the avian adipose tissue *in vitro* (1–3) also takes place *in vivo* in the species examined. An elevation of plasma FFA was observed in the goose with doses of 0.1 μg/kg. This amount of glucagon distributed in a volume of extracellular fluid

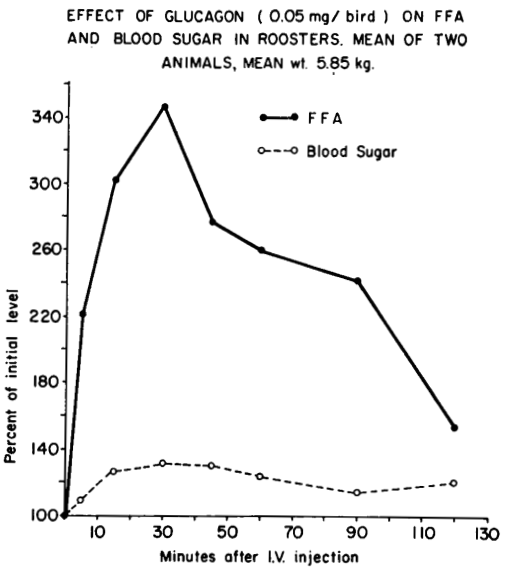

FIG. 1. Effect of glucagon injection on plasma FFA and blood sugar concentrations in roosters. Values expressed as percentage of the corresponding preinjection levels (means of 2 birds).

equivalent to 16% of the body weight will cause an increase of glucagon concentration of 0.6 μg/liter, which is about 60% of a recent estimate of the physiological concentration of glucagon (9). Sarcione *et al.* (10) have reported that glucagon produces an elevation of blood epinephrine levels in the dog. Because of the lack of effect of the catecholamines on the plasma FFA of the domestic fowl (1) it seems unlikely that the elevations of FFA reported here can be explained by liberation of epinephrine induced by glucagon, if such a phenomenon occurs in birds. The effect of glucagon on the plasma FFA of birds is in contrast with its effect in mammals since most of the reports indicate that glucagon produces a decrease of plasma FFA in man and dog (11–16). The elevation of blood sugar produced by glucagon in birds is in agreement with previous reports (17,18). The relative changes of blood sugar concentration, however, were smaller than the relative changes of FFA concentration in our experiments. This is particularly evident in the experiments with turkeys and roosters, as shown in Table IV and Fig. 1. The observations reported in this paper demonstrate that glucagon has a very marked effect in increasing plasma FFA in various avian species and support the possibility that glucagon plays a role in the process of fat mobilization in birds.

Summary. Intravenous injection of crystalline glucagon caused a prompt elevation of plasma FFA and blood sugar in geese, ducks, turkeys, and roosters. The FFA response in geese, for doses between 1 and 100 μg/kg showed a highly significant correlation with the logarithm of the dose ($r = 0.981$, $p<0.01$). The relative changes in FFA concentration, for a given dose of glucagon, were greater than the relative blood sugar changes. These observations indicate that glucagon may play a role in the process of fat mobilization in birds.

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