calf-kidney cultures, guinea pigs, and cattle (Table I).

Discussion. Since it is assumed that suspensions of genetically reproducible units are variable in a number of characteristics, it is not surprising to find that FMDV is variable in its sensitivity to heat. The application of heat to the wild strain of FMDV appears to destroy primarily the heat-sensitive particles. Thus, by the process of selection, heat-resistant particles remain. However, in the competitive reproduction of both, heat-sensitive particles appear to have a selective advantage in their replication for they tend to predominate in progeny. If this reversion process were to continue indefinitely in absence of heat treatment, it is expected that a complete reversion to the level of heat sensitivity of the wild type would be effected. Since the isolated resistant fraction—after 13 passages without heat—retains a significant resistance to the selecting agent, the heat treatment may effect a physical change within the virus particle itself. From work being reported elsewhere(Bachrach,3) it is clear that heating at 55°C denatures an infection-initiating property of the protein coat of FMDV without appreciable effect on its ribonucleic acid core.

Summary. A small thermal-resistant fraction (10^{-3} to 10^{-5} at 55°C) of foot-and-mouth disease virus, type A, has been isolated from a wild population. Under continuing thermal selection, it breeds populations which contain much higher fractions, at least 10^{-1}, of thermal-resistant virus particles. Such populations revert partially, however, toward the sensitivity of the wild strain if not subjected to heat.

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Detection of Insulin-Binding Antibodies and Separation of Free and Antibody-Bound Insulin by Rapid Chemical Procedure. (25589)

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The presence of insulin-binding antibodies in serums of insulin-treated patients has been demonstrated by paper strip chromato-electrophoresis, starch block electrophoresis and ultracentrifugation of insulin-I^{131} plasma mixtures(1), by cellulose column chromatography (2) and by precipitation of insulin-antibody complexes with rabbit antihuman globulin serum(3). These technics are all somewhat time consuming, and are not suitable for analysis of large volumes of material with low concentrations of radioactivity. It seemed worthwhile to attempt rapid separation of free and bound antibody-insulin by chemical procedure. Bates *et al.*(4,5) showed that in 76% ethanol saturated with NaCl most plasma proteins are precipitated at room temperature, while insulin remains largely soluble. The present paper describes adaptation of a modification of this procedure to separate free insulin from antibody-bound insulin.

Materials and methods. Control (non-immune) serums were obtained from subjects who had never been treated with insulin. Antiserums were obtained from insulin-treated patients. Donors of "immune" serums were not clinically insulin-resistant and required less than 75 units of insulin daily to control their diabetes. "Resistant" serums came from donors who required at least 150 units of insulin daily at the time their blood was drawn. All "immune" and "resistant" serums had been studied in detail previously(6). Insulin-I^{131} (specific activity, 38 - 73 mc I^{131}/mg insulin) and gamma globulin-I^{131} were prepared from

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crystalline beef insulin† and Cohn fraction II‡ respectively, by methods described previously (7). Human serum albumin-I^{131} was purchased from Squibb Labs. Paper-electrophoresis and hydrodynamic-flow chromatography were performed according to published methods(1). During preparation of insulin-I^{131} at high specific activity some labeled insulin undergoes radiation damage such as reductive splitting of the disulfide bonds(8) and binds non-specifically to plasma proteins, primarily to alpha-globulins(1). Results given below are corrected for damaged insulin.§ I^{131}-labeled proteins were incubated with serum at 37°C for 4 hours, then refrigerated at 4°C overnight. Fifty microliters of the mixtures were added to 1 ml of the various precipitating solutions. A white flocculent precipitate formed immediately in all cases and was separated immediately by centrifugation. Radioactivities in whole mixtures and precipitates were then assayed under similar geometrical conditions in a well-type scintillation counter. At the same time, aliquots of insulin-serum mixtures were analyzed for bound and free insulin by paper chromato-electrophoresis as described previously(1).

Results. NaCl-saturated 76% ethanol solution was in itself not suitable for separation of free and bound insulin at room temperature. Although more than half of albumin-I^{131} and almost all of gamma globulin-I^{131} were precipitated while 85% of insulin-I^{131} remained in solution, only 48% of antibody-bound insulin was precipitated (Table I).

Temperature and pH were found to influence considerably the solubility of free and antibody-bound insulin. In general, lower temperatures favored precipitability of insulin-antibody complexes more than that of free insulin whereas increasing acidity increased precipitation of free insulin (Table II). Accordingly, the best separations were obtained at high pH and low temperature. At −10°C and pH 6.6 - 8.0, over 90% of the antibody-bound insulin could be precipitated, but as much as 25% of free insulin-I^{131} was precipitated as well (Table II). On increasing the pH to 10.2 or above, precipitability of free insulin was decreased considerably without significant loss in precipitability of insulin-antibody complexes.

TABLE I. Precipitable Radioactivity in NaCl-saturated 76% Ethanol at 25°C pH 6.4.

	Control serum	Insulin-resistant serum*
Albumin-I^{131}	58%	
Gamma-globulin-I^{131}	94%	
Insulin-I^{131}	15%	48%

* 90% of insulin-I^{131} was bound to antibody in this serum as determined by paper electrophoresis.

The solution found to achieve the best separation was salt-saturated 76% ethanol in 0.01 M KOH. Following precipitation at −12°C to −10°C, (achieved in an ice-brine mixture) centrifugation was performed at 0°C since the small amounts of free insulin which coprecipitate at the lower temperature redissolve rapidly while insulin-antibody complexes remain as well-packed precipitates. Results of this procedure applied to the serums of 23 control subjects and insulin-treated patients are summarized in Table III. Precipitation of insulin-I^{131} in each case was compared with extent of insulin binding by antibody on

TABLE II. Precipitable Insulin-I^{131} in NaCl-saturated 76% Ethanol Solution.*

	0°C			−10°C
	pH 8†	pH 6.6‡	pH 3.5§	pH 8†
% insulin-I^{131} precipitated from control serum	10-11%	14-16%	27-31%	15-25%
% antibody-bound insulin precipitated from resistant serum	78-80	74-76	85-87	90-95

* Each set of values expresses range of 4 replicate determinations.
† .01M borate buffer.
‡ " phosphate " .
§ " acetate " .

† The Laboratory is indebted to Doctors Otto Behrens and C. W. Pettinga of Eli Lilly Research Labs. for generous supplies of crystalline insulin.

‡ Prepared by Dr. Eric Pollaczek.

§ This damage generally amounted to about 10-15% of total radioactivity as determined by paper chromato-electrophoresis of insulin-I^{131} in control serum. In antiserums, damaged fractions which bind to α and β-globulins and to albumin are readily distinguished from antibody-bound insulin which migrates at front of gamma-globulin zone(1).

TABLE III. Precipitation of Insulin-I^{131} from Incubated Mixtures of Insulin-I^{131} and Serum by NaCl-saturated 76% Ethanol Solution in 0.01 M KOH at −12°C.

Serum dilution	Type	% radioactivity precipitated	% radioactivity bound to Ab by chromato-electrophoresis	% Ab-bound insulin precipitated
Undil.	Control	7	None	
		15	"	
		6	"	
		6	"	
		6	"	
	Immune	51	82	62
		75	86	87
		63	71	89
		55	85	65
		90	91	90
		57	89	64
		75	75	100
		96	95	101
		89	90	99
		87	90	97
		85	90	95
		76	84	90
1:10	Resistant	86	90	91
"		70	79	89
"		87	90	96
1:50		78	90	87
1:10		91	90	101
1:50		80	80	100

paper chromato-electrophoresis. No more than 15% of free insulin was precipitated from control serums, while in 15 of 18 antiserums over 87% of the bound insulin was precipitated.

A large number of experiments was performed in which salt and ethanol concentrations were varied. In general, decrease in salt concentration or increase in alcohol concentration favored precipitation of free insulin with the serum proteins. With the modifications of temperature and pH described, the medium of Bates *et al.*(4,5) was found to be the most effective for the present purpose.

Summary. A rapid chemical method for detection of insulin-binding antibodies and separation of free and antibody-bound insulin in human antiserums is described. With NaCl-saturated 76% ethanol solution in 0.01 M KOH at −12°C to −10°C, 87% - 100% of antibody-bound insulin was precipitated from 15 of 18 insulin antiserums whereas 85% - 94% of unbound insulin in control serums remained in solution.

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Effect of Phentolamine (Regitine®) on Human Plasma FFA *in vivo*.* (25590)

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Studies of plasma free fatty acid (FFA) levels in man and animals suggested that the sympathetic division of autonomic nervous system affects lipid metabolism(1). Administration of noradrenalin and adrenalin causes rapid rises in plasma FFA levels. Administration of an autonomic ganglion blocking

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