probable that such inhibition may be caused by the reaction between IgG antibodies bound to cell cultures and the antibodies to gamma globulin; at high serum concentrations this reaction may result in complete covering of antigenic sites of IgG, which prevents the binding of indicator erythrocytes. Future experiments will have to show whether antibodies demonstrable by mixed agglutination may play some pathogenic role. This would seem conceivable since these antibodies are directed against "accessible" antigens of the cell surface. However, before any speculation along this line can be made, evidence has to be presented that these antibodies are autoantibodies. To this end, studies performed on cultures of the patient's own cells will be necessary. Thus far, we did not succeed in obtaining such material.

Summary. Eleven sera of patients with bullous skin diseases were studied for antibodies to cell surface antigens by means of mixed-agglutinatioon tests with cell cultures. It was shown that seven sera contained IgG antibodies reacting with epithelial cell cultures but none of them had antibodies combining with fibroblastic cell cultures. The antibodies reacting with cell cultures were different from antibodies to intercellular antigens of epidermis detectable by immunofluorescent staining technique. Besides these antibodies, some sera of patients with bullous skin diseases were shown to contain IgM antibodies with anti-gamma globulin activity resembling the rheumatoid factor.

The authors wish to acknowledge the technical assistance of Mrs. Margaret Grybel and Mrs. Nancy Brummitt.

1. Beutner, E. H. and Jordon, R. E., Proc. Soc. Exptl. Biol. Med., **117**, 505 (1964).
2. Beutner, E. H., *et al.*, J. Am. Med. Assoc., **192**, 682 (1965).
3. Milgrom, F., *et al.*, J. Immunol., **92**, 8 (1964).
4. Karzon, D. T., *et al.*, Virology, **9**, 564 (1959).
5. Milgrom, F., *et al.*, J. Am. Med. Assoc., **192**, 845 (1965).
6. Milgrom, F., *et al.*, J. Am. Med. Assoc., **198**, 226 (1966).
7. Kano, K., and Milgrom, F., Intern. Arch Allergy Appl. Immunol., **31**, 209 (1967).
8. Waller, M. V. and Vaughn, J. H., Proc. Soc. Exptl. Biol. Med., **92**, 198 (1956).
9. Milgrom, F. and Witebsky, E., J. Am. Med. Assoc. **174**, 56 (1960).
10. Deutsch, H. F. and Morton, J. I., Science, **125**, 600 (1957).
11. Brakke, M. K., Arch. Biochem. Biophys., **55**, 175 (1955).

Received Sept. 5, 1967. P.S.E.B.M., 1968, Vol. 127.

Cold Stimulation of Organs in Relation to Potassium Concentration and Drug Action (32690)

JUAN ARBONA I. (Introduced by N. Ercoli)
Escuela de Medicina "José Vargas," Cátedra de Farmacologia, Universidad Central de Venezuela, Caracas

The different reactivity of various organs to thermal stimulus (1) led us to investigate more closely the correlation between cold contraction and K^+ concentration, for a better understanding of the mechanism involved. With the same purpose, we studied the effect of various pharmacological agents on cold contraction and K^+ liberation.

It has been established already (2) that cold stimulation of smooth muscles induces intracellular variations of K^+ concentration which appear also in the perfusion fluid.

Materials and Methods. Segments (3–4 cm) of colon and vas deferens of white rat, and guinea pig colon were used. The organs were suspended in tissue bath containing 15 ml of Tyrode's solution, maintained at a temperature of 37°C and oxygenated with a mixture of 95% O_2 and 5% CO_2. The composition of the Tyrode's solution (in gm/liter of distilled water) was NaCl 8.0; KCl 0.2; $CaCl_2 \cdot 2H_2O$ 0.26; $MgCl_2 \cdot 6H_2O$ 0.21; NaH_2

$PO_4 \cdot 2H_2O$ 0.06; $NaHCO_3$ 1.0; and glucose 1.0.

The isotonic contractions were registered kymographically through a frontal writing lever, with a load of 1 gm giving a 1:10 magnification. The drugs used were norepinephrine bitartrate; 5-(3-methylaminopropyl)-5*H*-dibenzo (*a,d*) cycloheptene hydrochloride (Protriptyline), reserpine ascorbate and "sodium-free Tyrode" (3). The cold stimulus was obtained by cooling the bath from 37 to 4°C. Each determination is based on 9 experiments.

Results. (a) *Guinea pig colon.* The K^+ induces contraction of guinea pig colon at concentrations of 0.029 meq/ml. Subliminal quantities of K^+ (0.014 meq/ml) added to the perfusion fluid of the organ cooled from 37 to 4°C do not modify the contraction induced by cold stimulation. Increasing K^+ concentrations, even to maximal contractive effectiveness, the additional cold stimulus does not exceed its original response (Fig. 1). Observing the form of the contraction recordings, it would appear that the total response is the resultant of the K^+ effect completed by the cold stimulus (until its original maximal effectiveness).

(b) *Colon and vas deferens of rat.* In contrast to the findings in guinea pig, subliminal amounts of K^+ added to the organ, followed by cooling, induce a response which is higher than that of the controls exposed to cold stimulation alone. The addition of 1 ml of Tyrode's containing 0.292 meq/ml of K^+ to the bath induces no contraction of vas deferens, while in the presence of the same K^+ amount, the cold induced contraction increases from 2.88 ± 0.32 mm to 14.16 ± 2.35 mm (Fig. 2), indicative of a high degree of potentiation determined by K^+.

In the organs of both species, the cold contraction no longer takes place when Na^+ is completely replaced by K^+. Thus, in order to maintain the influence of cold on these systems, a certain amount of free sodium has to be present.

Since cold contraction induces variations of membrane potential (4) and K^+ content (5), we thought that drugs which potentiate pharmacological effects by acting on membrane (6) may influence the cold effect also. We have selected Protriptyline, a dibenzazepine (acting centrally as an antidepressive drug)

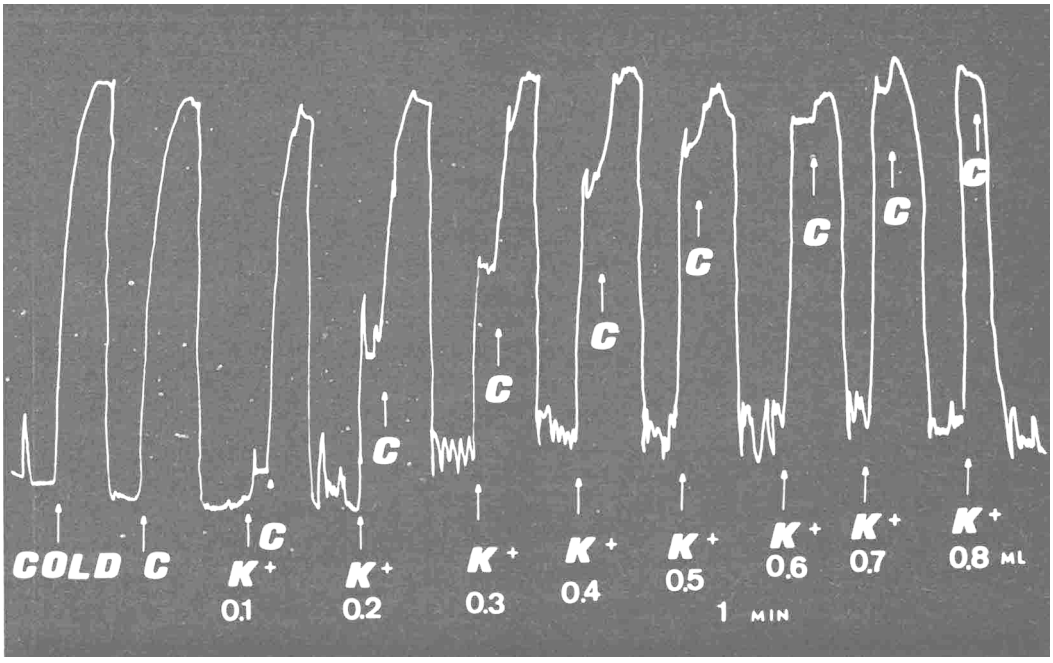

FIG. 1. Influence of K^+ addition on cold contraction.

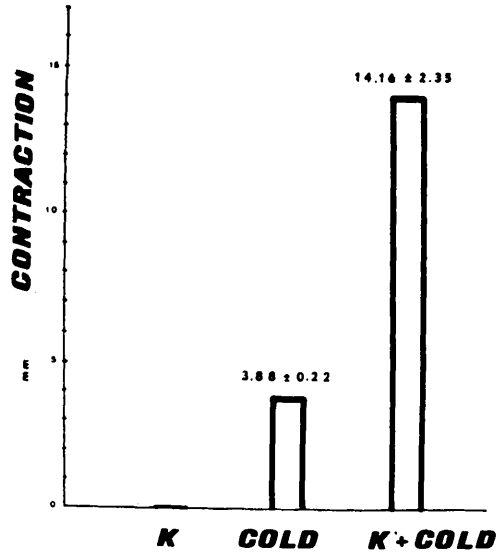

FIG. 2. Synergism between cold stimulation and potassium addition on rat vas deferens.

which potentiates norepinephrine by its peripheral effects on the vegetative system (7). In fact, we found that Protriptyline (Fig. 3) sensitizes rat vas deferens to thermal stimulation and to K^+, as well as to norepinephrine. Characteristically, Protriptyline by itself causes relaxation of the vas deferens (but less than of the rat colon) while its potentiating effect on K^+ contraction in some experiments reached 500%.

The known antagonistic effect of reserpine on a number of agonists acting on smooth muscle (8), led us to investigate its influence on cold contraction. Intraperitoneal reserpine doses of 0.003 mg/kg in the guinea pig, made the isolated intestine of the animal, sacrificed after 1 day, totally insensitive to cold stimulus.

The same results were obtained in rats which received 5 mg/kg of reserpine intraperitoneally.

The isolated intestine of rats and guinea

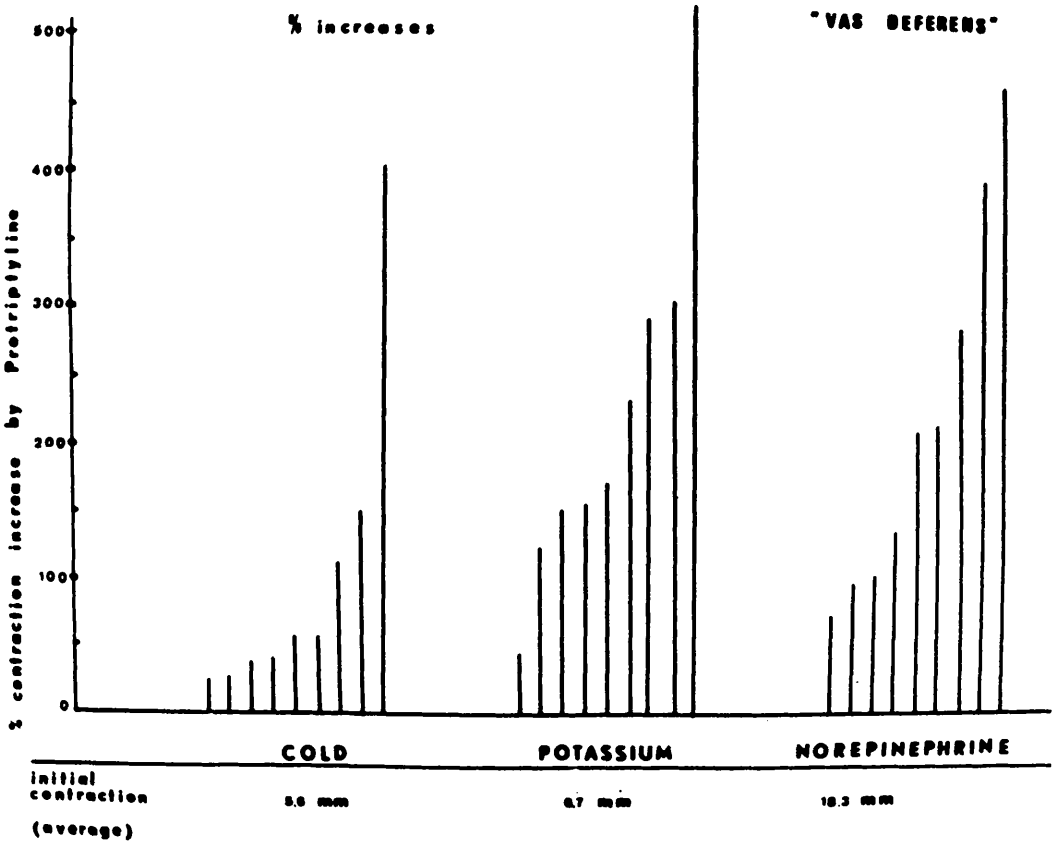

FIG. 3. Protriptyline potentiation of cold, K^+, and norepinephrine contractions of the rat.

pig treated with reserpine *in vitro* behaved in a qualitatively different manner. Reserpine concentrations of 3.33 μg/ml inhibited completely the cold contraction of the guinea pig intestine, which remained relaxed. Yet, by repeated cold stimulation this organ became sensitive again. On the isolated intestine (and vas deferens) of rat, up to 20 μg reserpine did not inhibit cold contraction and only the spontaneous movements of the organs were abolished (Fig. 4).

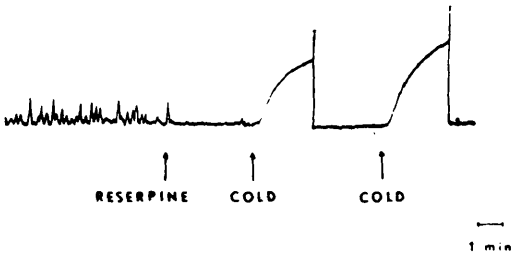

FIG. 4. Effect of reserpine on spontaneous movements of rat intestine and on cold contraction. (Reserpine abolished the cold contraction of guinea pig intestine.)

For an analysis of the mechanism of action, we have added to the guinea pig intestine maintained at 37°C, reserpine for a period of 15 min and determined K concentrations, comparing it with that of organs maintained for 3 hours at low temperature (4°C) in the presence of the drug. At the same time, organs not exposed to reserpine were observed for variations of K^+ concentration. The significant results of these experiments are given in Table I.

At 37° reserpine treated organ baths showed the same K^+ concentrations as the controls, while cooling in the presence of reserpine gave a significant K^+ increase ($p = .005$) over the cold controls.

Conclusions. (i) The effect of cold stimulation is differently influenced by potassium in various species. In the isolated smooth muscle of guinea pig the effect of cold and K^+ is additive within limits (Fig. 1) and represents a summation with degradation, while in the rat a potentiation takes place. The latter effect has been observed by Guttman and Ross (4) on the smooth muscle of Mytilus edulis. The underlying mechanisms responsible for these differences could be attributed to: basal variations in the depolarization process; equilibrium conditions between extrinsic K^+ and K^+ released by the cold stimulus; muscular contractility depending on other processes than those related to depolarization.

(ii) Protriptyline potentiates the effects of cold, K^+, and of norepinephrine on the smooth muscle. Since norepinephrine induces a hyperpolarization and cold and K^+ a depolarization, it would appear that Protriptyline acts in a nonspecific manner on hyperpolarizing and depolarizing agents, which presumes a common component, influencing, or being influenced, in both mechanisms.

(iii) Temperature decrease causes a contraction of guinea pig intestine with liberation of K^+; the addition of reserpine inhibits cold contraction, though it increases K^+ liberation by 32.7%. Troquet and co-workers (9) showed that intracellular K^+ liberation takes place in the myocardium of rats treated with reserpine, causing cardiac insufficiency. Possibly the cardiac contraction is essential for the increase of K^+ liberation in presence of

TABLE I. Effect of 3.33 μg/ml of Reserpine on Potassium Concentration in the Perfusion Liquid of Guinea Pig Colon after 3 Hours' Contact.
(2 gm of intestine in 15 ml of Tyrode's)

	1	2	3	4
Perfusion liquid	Tyrode's alone	Tyrode's with reserpine	Tyrode's alone	Tyrode's with reserpine
Temperature	37°C	37°C	4°C	4°C
K^+ (meq)	2.8 ± 0.52	2.7 ± 0.66	5.11 ± 1.05	6.63 ± 1.22
K^+ increase (%) over 37° control	0	0	93	145
p	between 1, 2 and 3: .000004		between 3 and 4: .005	

reserpine, a supposition which deserves comparative experiments with myocardial fiber preparations at rest.

Summary. Analyzing the mechanism of thermal reaction, we found that exogenous K^+ potentiates cold contraction in rat intestine (or vas deferens) but has no similar effect on guinea pig intestine. A dibenzazepine, Protriptyline, which slightly relaxes the vas deferens of rat, induces a high potentiation of the contractions by thermal stimulus, K^+, and norepinephrine. Norepinephrine contraction is not modified by thermal stimulation or K^+. Reserpine is species specific on the isolated intestine: it abolishes cold contraction in guinea pig, while in rats only the spontaneous movements are decreased. However, the thermal response of the intestine in both species is inhibited by *in vivo* reserpinization. Cooling of guinea pig intestine in presence of reserpine increases K^+ release, a mechanism which may be responsible for some of its toxic action on contracting organs, such as the cardiac insufficiency in rats accompanied by K^+ release (9) and in patients treated with cardiac glycosides and reserpine (10).

1. Ercoli, N. and Guzzon, J., Science **115**, 672 (1952).

2. Daniel, E. E., Ann. Rev. Pharmacol. **4**, 189 (1964).

3. Evans, D. H. L., Schild, H. O., and Thesleff, S., J. Physiol. **143**, 474 (1958).

4. Guttman, R. and Ross, S. M., J. Gen. Physiol. **42**, 1 (1958).

5. Daniel, E. E., Can. J. Biochem. Physiol. **41**, 2065 (1963).

6. Flemming, W. W., Intern. Pharmacol. Congress, 3rd, Sao Paulo, 1966.

7. Cairncross, K. D., McCulloch, M. W., and Mitchelson, F., J. Pharmacol. Exptl. Therap. **149**, 365 (1965).

8. Gills, C. N. and Lewis, J. J., J. Pharm. Pharmacol. **20**, 606 (1956).

9. Troquet, J., Colinet-Lagneaux, D., and Hermann-Gedang, I. Arch. Intern. Pharmacodyn. **163**, 232 (1966).

10. Lage, G. L. and Spratt, J. L., Proc. Soc. Exptl. Biol. Med. **125**, 580. (1967).

Received Sept. 5, 1967. P.S.E.B.M., 1968, Vol. 127.

Differential Accumulation of Glycine by Avian (Chicken) and Mammalian (Human) Erythrocytes: Lack of Insulin Effect* (32691)

GLORIA BISESI AND MERVYN C. BERMAN (Introduced by I. L. Schwartz)
Medical Research Center, Brookhaven National Laboratory, Upton, New York 11973 and Mount Sinai Medical and Graduate Schools, New York, New York 10029.

Significant differences have been found in the accumulation of glycine by avian erythrocytes and mammalian erythrocytes. In the former the glycine concentration is 3–5 times higher than that of the plasma (1), while in the latter it is approximately equal to that of the plasma (1,2,3). While amino acid incorporation into avian erythrocytes is diminished by the presence of metabolic respiratory inhibitors such as cyanide, arsenate, and dinitrophenol, these agents do not affect the accumulation of glycine in mammalian erythrocytes (1).

* This work was supported by the U. S. Atomic Energy Commission and U.S. Public Health Service Grant No. AM-10080 of the National Institute of Arthritis and Metabolic Diseases.

In the course of experiments on the relationship of amino acid transport and protein synthesis, we have studied the effect of insulin on the uptake of glycine by nucleated erythrocytes, which are capable of protein synthesis (4)—as compared with nonnucleated erythrocytes, which are not capable of protein synthesis (2,3). We found that the nonnucleated cells accumulated glycine at a more rapid rate than did the nucleated cells, but the transport of this amino acid proved to be insensitive to insulin in both cell types.

Freshly heparinized whole human or chicken blood was centrifuged and the plasma decanted. The cells were suspended five times in 50–100 volumes of 0.9% NaCl at 4°C. and