Substance Q: A Smooth Muscle Contracting Substance Liberated from Human Plasma Lipids by the Action of Heat

A substance in the G.2 fraction of human blood plasma with a slow contracting activity on smooth muscle (S.M.C.) has been isolated and designated as G acid[1]. Another substance which gives a quick contraction of plain muscle was produced during the isolation procedure. It is formed by heating the G.2 fraction to 150 °C for a short time. This quick muscle-contracting activity (Q activity) has been concentrated and its properties studied.

Methods. The precipitation of the G.2 fraction from citrated plasma was carried out after the separation of fibrinogen and prothrombin by the ether method devised by KEKWICK and MACKAY[2]. G.2 solution (60 ml of 4.8% solution in 0.85% saline), cooled to 0 °C, was added gradually and with constant stirring to 360 ml of alcohol-ether mixture (7:3 v/v) at − 10 °C. After standing for 1 h the precipitate was removed by centrifugation at 0 °C and the supernatant fluid was evaporated to dryness under reduced pressure, dried in a vacuum over P_2O_5 and extracted with A.R. pentane (b.p. 25–40 °C). When 437 mg of the crude pentane extract were subjected to vacuum sublimation at 200 °C and 10^{-3} mm pressure for 5 min, 228 mg of residue (R_{200}) were obtained. This residue contained both the S.M.C. and Q substances. The 2 substances could be separated from each other by dialysis through cellophane against distilled water, substance Q being readily dialysable.

Concentration and purification of Q activity. A 7-step purification procedure has been developed to separate substance Q. This can bring about a 10,000–20,000 fold concentration of the Q activity with a final recovery of 10% of the active material. Starting with the G.2 fraction, the purification procedure consisted of:

(1) *Dialysis of G.2.* Sterile G.2 fraction (10% suspension in distilled water) was dialysed in a cellophane bag against distilled water for 1 h at 1–2 °C, using a magnetic stirrer to speed the dialysis process. This process was repeated twice. In this connection, it is important to note that the precursor of substance Q is not dialysable under these conditions, since it was found that dialysis does not influence the release of substance Q from G.2 fraction. The dialysed material was then freeze-dried.

(2) *Heat treatment of the dialysed material.* The freeze-dried product was then heated in a pyrex flask dipped in an air bath maintained at a temperature varying from 150 °C to 180 °C for 15 min. The flask was covered with a loose watch glass in order to minimize the loss of Q activity through atmospheric oxidation.

(3) *Dialysis.* The product obtained after heat treatment was then vigorously shaken with distilled water (minimum amount to form a paste) and dialysed in a cellophane bag against an equal volume of distilled water for 1 h at 1–2 °C, using a magnetic stirrer. Dialysis was repeated twice. The combined diffusates were then evaporated to dryness under reduced pressure in a nitrogen atmosphere and below 40 °C, and stored over P_2O_5 in vacuo.

(4) *Alcohol extraction.* The dried material obtained in step 3 was then extracted with three 5 ml portions of hot absolute ethanol, and the combined extracts were evaporated to dryness.

(5) *Partial sublimation of the alcohol extract in vacuo.* The alcohol extract was then subjected to partial sublimation at 150 °C under a vacuum of 10^{-3} mm. About 50% by weight of the inactive substances could be sublimed by this treatment. The residue was extracted with hot absolute ethanol (1 ml per 100 mg) and the insoluble material discarded. The ethanolic solution was then evaporated down to dryness.

(6) *Chromatography.* The dried alcohol extract obtained in step 5 was dissolved in absolute methanol (1 ml per 100 mg). The solution was filtered through a Savory and Moore alumina column (15 · 1 cm) previously washed with 100 ml of absolute methanol. The column was then developed with absolute methanol, the first effluent (10–15 ml) was discarded and the next yellowish fraction (about 15 ml) collected. This fraction, which contained about 90% of the active principle from the previous step, was evaporated to dryness.

(7) *Fractionation with absolute ethanol.* The active material obtained in step 6 was soaked in absolute ethanol (0.1 ml per 10 mg). The insoluble fraction, separated by centrifugation, stimulated the isolated guinea-pig jejunum in a dose of 1 μg.

Starting with 100 g of G.2 fraction, 0.5 mg of this potent material was obtained. The Table gives the concentration and recovery of substance Q at the various steps of purification described above. In this Table, 1 unit of substance Q is defined as the quantity which causes, in the isolated guinea-pig jejunum suspended in a 10 ml bath, a quick contraction similar to that obtained with 20 mg of G.2 after heat treatment.

Results and discussion. The purest preparation of substance Q is a white amorphous powder, freely soluble in water, methanol and ethanol (less soluble in ethanol than in methanol), but practically insoluble in ether or pentane. The optical rotation of the methanol solution (1 mg/ml) is negligible. The UV-absorption spectrum of the methanol solution shows a maximum at 2200 Å. The active principle is easily dialysable and is relatively heat stable. It is destroyed, however, by prolonged heating in air above 150 °C. It is not destroyed by an esterase-rich fraction prepared from human serum[3].

The sodium fusion test on the purified substance shows the presence of nitrogen and the absence of sulphur and halogen. The active principle is not precipitated from aqueous solutions by trichloroacetic acid. Both Biuret and Molisch's tests are negative. These data suggest that the active principle of the Q activity is not a protein, a polypeptide or a carbohydrate. The % of nitrogen in the purified substance as determined by the micro-Kjeldahl

Separation of substance Q from G.2 fraction of human blood plasma

Step	Original G.2 recovery (U/g)	Dry weight (U/g)
2	50	50
3	40	5,000
4	26	14,000
5	11	20,000
6	10	200,000
7	5	1,000,000

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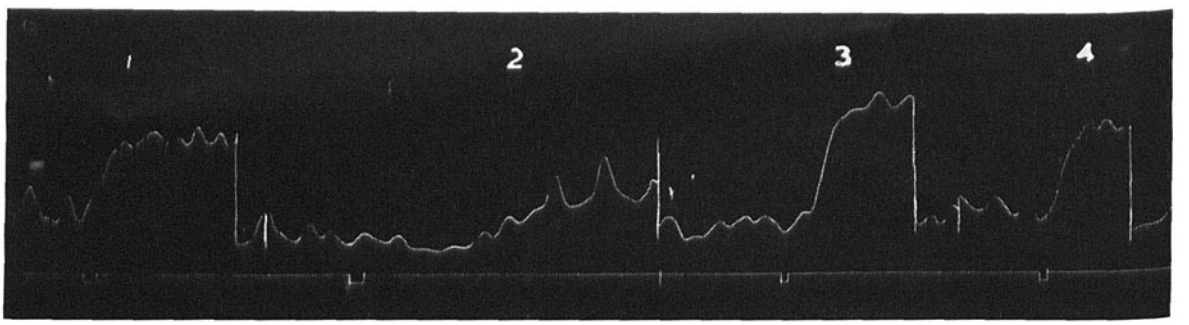

Fig. 1. Effect of substance S.M.C. and substance Q on the isolated guinea-pig jejunum. Responses to (1) 10 mg of the residue R_{200} (substance S.M.C. and substance Q), (2) 20 mg of the pentane extract of G.2 fraction (substance S.M.C.), (3) 0.2 µg histamine dihydrochloride and to (4) 10 mg of the residue R_{200}.

method is 12.2. Phosphorus estimated by the method of MARTLAND and ROBINSON[4] gave 0.04% for inorganic P, and 0.18% for the total P, both values are probably of no consequence.

Some pharmacological aspects of substance Q. Substance S.M.C. causes contraction of the isolated guinea-pig jejunum after a latent period varying from 10–20 sec, depending on the sensitivity of the preparation. After the pentane extract of substance S.M.C. had been in contact with the preparation for 30 sec, the preparation was washed by allowing the test fluid to flood over the rim of the organ bath. The contraction persisted after washing, slowly returning to the base line. This took from 1–2 min. In the case of substance Q, the smooth-muscle contraction was rapid (histamine-like) and the preparation was washed after the lever had reached the summit of its movement (Figure 1).

Preparation (a) of substance Q is active on the isolated guinea-pig jejunum in a dose of 2 µg in a 10 ml bath (Figure 2). It is as potent as 5-hydroxytryptamine. Preparation (b) is active in a dose of 10 µg on the same muscle preparation. Preparations (a) and (b) are obtained after the 7th and 6th steps of the purification procedure, respectively.

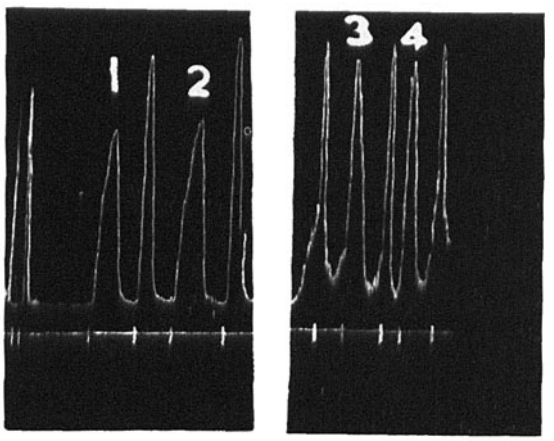

Fig. 2. Effect of substance Q and 5-hydroxytryptamine on the isolated guinea-pig jejunum. Responses to (1) 10 µg of preparation (b), (2) 2 µg of preparation (a), (3) 2 µg of 5-hydroxytryptamine, and to (4) 2 µg of preparation (a). Other contractions are due to 0.2 µg histamine dihydrochloride. (N.B. – Standard solution of 5-hydroxytryptamine was prepared from the complex 5-hydroxytryptamine creatinine sulphate which contains 43.5% of the active base.)

The properties of substance Q resemble those of a choline ester, except that it is not destroyed by blood cholinesterase. Substance Q is not choline because the finally purified material is about 100 times more active than choline on the isolated guinea-pig jejunum. Substance Q is not acetylcholine, because it is not destroyed by the esterase-rich fraction prepared from human serum which destroys acetylcholine under similar conditions, indicating further that it is probably not a choline ester. Also, substance Q cannot be sublimed under the same conditions which sublime acetylcholine hydrochloride. Substance Q is not phosphorylcholine, because the finally purified material is practically free from phosphorus. It is not histamine, because histamine does not cause contraction of the rat's colon and rabbit's jejunum which normally respond to substance Q. Moreover, the effect of the substance is not antagonized by antistin. The fact that substance Q is atropine sensitive distinguishes it from substance P[5] and substance R[6]. Substance Q differs from substance R and such proteolytic enzymes as renin, trypsin and chymotrypsin, since it is dialysable and relatively heat stable. Substance Q is not 5-hydroxytryptamine, because the finally purified material gives negative Hopkins-Cole, Ehrlich and Folin tests (the colour tests of 5-hydroxytryptamine). Besides, the active material at pH 3.5 does not show a maximum absorption at 2750 Å, as in the case of 5-hydroxytryptamine[7], although 5-hydroxytryptamine and the purest preparation of substance Q show similar activity on the isolated guinea-pig jejunum (Figure 2).

Résumé. Sous l'effet de la chaleur, la substance Q qui cause la subite contraction des muscles non soumis à la volonté, se détache des matières grasses qui se trouvent associées à la fraction G.2 du plasma humain. Nous avons réussi à concentrer cette substance 20000 fois.

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