THE EFFECT OF SOME METALS, VITAMINS, ANÆSTHETICS, AND OTHER SUBSTANCES ON UNSTRIATED MUSCLE

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PART I

Action of Heavy Metals

Many metals are indispensable for life in small concentrations. They are present in the food we eat and in the water we drink and are constituents of protoplasm. Every living organism without exception contains the following elements: carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, chlorine, potassium, sodium, calcium, magnesium, copper and iron. In addition to these thirteen elements, others are found in certain organisms, sometimes to a large extent. Of these elements we may mention especially silicon, iodine, fluorine, bromine, aluminium, nickel, cobalt, zinc, lead, silver, cadmium, lithium, strontium, and manganese (Fox and Ramage, 1931).

In the present research the action of the following metals on the excitability of unstriated muscle was investigated: arsenic trichloride, uranium nitrate, antimony chloride, bismuth subnitrate, ferric chloride, mercuric chloride, chromium chloride, aluminium chloride, silver nitrate, gold chloride, platinum chloride, copper sulphate, zinc chloride, cadmium chloride, cobalt chloride, nickel chloride, manganese chloride, and lead nitrate. Many of these metals are precipitated by alkaline Ringer solution (pH 8, borate), or by the chlorides but in the concentrations used (I in 10^7–10^4), they were found to be sufficiently soluble to exert definite actions. The action was that of the metal, as the corresponding anions in the concentrations used had no significant effect. The muscle used was mostly that of the frog stomach and was stimulated by alternating current (A.C.), direct current (D.C.), and potassium (0·02 to 0·04 M KCl).

Action of zinc.—Traces of zinc are indispensable for nutrition. Salant and Mitchell (1916) found that the movements of the excised intestines were depressed by very dilute solutions and abolished by higher concentrations. 1 in 10^7 improves the response to A.C., D.C., potassium and acetylcholine in the dog stomach. In the frog stomach it improves the response to A.C.,

D.C., potassium but higher concentrations are depressant. Higher concentrations, 1 in 10^{6}–10^{4} increase the response to potassium and decrease that to electric current, showing a differential effect on the two excitabilities. 1 in 10^{6}–10^{5} increase the response to acetylcholine in dog stomach, but higher concentrations depress it. 1 in 10^{4} causes tonic contraction and depresses excitability in general and appears to be toxic.

Action of mercury.—1 in 10^{7} improves the response to A.C., D.C., in dog and frog stomachs, but depresses that to potassium; 1 in 10^{6} has opposite effects. In dog stomach 1 in 10^{7} depresses and 1 in 10^{6} improves the response to acetylcholine. 1 in 10^{5} is depressant and causes tonic contraction.

Action of cadmium.—The rest of the experiments were performed on the frog stomach. 1 in 10^{7}–10^{6} inhibit tone, decrease the response to potassium, increase that to A.C. and D.C. 1 in 10^{5} increases and 1 in 10^{4} decreases tone; these concentrations are depressant to A.C. and potassium.

Action of copper.—1 in 10^{7} inhibits tone and the response to potassium, but greatly improves the response to A.C. 1 in 10^{6} decreases tone and response to A.C., but increases that to potassium. Higher concentrations cause tonic contractions and are depressant to alternating current and potassium.

Action of silver.—1 in 10^{7} decreases tone and response to A.C., but increases that to potassium. 1 in 10^{6} decreases the response to A.C., potassium and tone; 1 in 10^{5} has the opposite action. 1 in 10^{4} causes tonic contraction and decreases the response to A.C., and potassium.

Action of gold.—1 in 10^{7} improves the response to A.C., and potassium but inhibits tone. 1 in 10^{6} decreases the response to A.C., but increases that to potassium; 1 in 10^{5}–10^{4} has opposite action. 1 in 10^{3} is depressant to A.C., and potassium, but causes tonic contraction.

Action of aluminium.—1 in 10^{7} increases the response to A.C., and potassium, but diminishes tone; 1 in 10^{6} decreases all. 1 in 10^{5}–10^{4} causes tonic contraction.

Action of tin.—1 in 10^{7}–10^{5} increases the response to A.C., and decreases that to potassium; 1 in 10^{4} has the opposite action and increases tone.

Action of lead.—1 in 10^{7} increases the response to A.C., and potassium and decreases tone. 1 in 10^{6}–10^{4} increases the response to A.C., and decreases that to potassium and tone.

Action of arsenic.—1 in 10^{7} increases the response to A.C., and potassium but diminishes tone. 1 in 10^{6}–10^{5} decreases all. 1 in 10^{4} causes tonic contraction but depresses excitability.

Action of antimony.—1 in 10^7 increases the response to A.C., and potassium but diminishes tone. 1 in 10^6 depresses the response to A.C. but increases that to potassium and diminishes tone. 1 in 10^5 depresses all. 1 in 10^4 causes tonic contraction.

Action of bismuth.—1 in 10^7 increases the response to A.C. and potassium but decreases tone. 1 in 10^6 decreases the response to A.C. but increases that to potassium and tone. 1 in 10^5–10^4 depresses the response to A.C., and potassium but causes tonic contraction.

Action of chromium.—1 in 10^6 increases the response to A.C. and potassium. Higher concentrations (1 in 10^6–10^4) are depressant and decrease tone.

Action of uranium.—1 in 10^7 increases the response to A.C. and potassium but decreases tone. 1 in 10^6 increases the response to A.C. but decreases that to potassium and tone. 1 in 10^5 decreases the response to A.C. and increases that to potassium but diminishes tone. 1 in 10^4 is depressant.

Action of manganese.—1 in 10^7 increases the response to A.C. and potassium; 1 in 10^6 increases the former and decreases the latter. Higher concentrations (1 in 10^5–10^4) depressant.

Action of iron.—1 in 10^7 improves the response to potassium. 1 in 10^6 decreases the response to both and tone. 1 in 10^5 decreases the response to A.C. but increases that to potassium and decreases tone. 1 in 10^4 has the opposite effect on response to A.C. and potassium.

Action of cobalt.—1 in 10^7 decreases the response to A.C. and increases that to potassium and tone. 1 in 10^6 decreases the response to A.C. and potassium but increases tone. 1 in 10^5 has similar action. 1 in 10^4–10^3 is depressant and decreases tone.

Action of nickel.—1 in 10^7–10^5 decreases the response to A.C., but 1 in 10^4 increases it. Salant and Mitchell (1916) found that dilute solutions of nickel salts produce depression which may be followed by stimulation. 1 in 10^7–10^4 increases the response to potassium. 1 in 10^7–10^5 increases and 1 in 10^4 decreases tone.

Action on inhibition.—Zinc antagonises the inhibition produced by adrenaline (1 in 10^6) in the guinea pigs uterus in concentrations 1 in 10^7–10^4, but augments the potassium inhibition. It augments one kind of electrical inhibition and antagonises the other. If tonic contraction develops then it may antagonise all kinds of inhibitions. This is in agreement with the fact that protein precipitants may change an inhibitory into an excitatory effect (Sollmann, 1942).

Discussion

Many poisonous metals are beneficial in small concentrations; this reminds one of the Ayurvedic System of medicine. There is some relation between the electronic configuration of atoms and the physiological action. Thus in Groups I and II of the Periodic Table, the elements of subgroup *a* are comparatively less toxic than those of the subgroup *b*. Thus lithium, sodium and potassium are less toxic than copper, silver and gold. Similarly magnesium, calcium, strontium, and barium are less toxic than zinc, cadmium and mercury.

Toxic doses of metals cause tonic contraction. This is in agreement with the view mentioned previously that anything that injures the cell membrane would cause tonic contraction (Singh, 1944*b*). In small concentrations they cause inhibition. This is perhaps related to the action of some of them in destroying parasites *in vivo*. It is possible that they produce inhibition of some activity of the parasites and so make them susceptible to other agencies of the defence mechanism of the body. Thus it is known that *in vivo* these metals destroy parasites in much smaller concentrations than *in vitro*.

On unstriated muscle, the action of metals resembles those of other substances. Thus they may increase the excitability or decrease tone and *vice versa*, or they may differentially affect the excitability to A.C. and potassium and tone in an independent manner (Singh, 1944*a*).

PART II

Action of Water-Soluble Vitamins

Action of thiamine.—

In frog stomach.—1 in 10^7 increases the response to alternating current and spontaneous contractions, and does not significantly affect the response to potassium and tone. 1 in 10^6 then decreases the response to A.C. and increases that to potassium; tone decreases in muscles which are sensitive to electric current, but increases in those sensitive to potassium. Further concentrations (1 in 10^5–10^3) are depressant, but tone is affected as above.

In dog stomach.—1 in 10^7 greatly increases tone, so that the excitability is depressed both as regards A.C. and potassium. With 1 in 10^6, tone decreases, so that the response to A.C. slightly improves; this action increases with 1 in 10^5. 1 in 10^4 again increases tone. Small concentrations (1 in 10^7–10^6) thus cause contraction, and larger concentrations (1 in 10^5) relaxation.

Action of riboflavine.—

In frog stomach.—1 in 10^7 increases the response to A.C., and does not significantly affect that to potassium and tone; 1 in 10^6 then decreases the response to A.C. Higher concentrations (1 in 10^5–10^4) are depressant and decrease tone.

In dog stomach.—The action resembles that in the frog stomach. Small concentrations increase and large concentrations decrease tone.

Action of nicotinic acid.—

In frog stomach.—1 in 10^7 has no significant action. 1 in 10^6–10^5 increases the response to A.C. and potassium and does not significantly affect tone. 1 in 10^4 is depressant and decreases tone.

In dog stomach.—1 in 10^7 increases tone and decreases the response to potassium. 1 in 10^6 increases the response to A.C., potassium and tone. 1 in 10^5 then decreases the response to A.C., increases that to potassium and decreases tone. 1 in 10^4 was found to increase the response to A.C. but decrease the response to potassium and tone.

Small concentrations thus increase and large concentrations decrease tone, as a result of which the response to A.C. increases.

Action of ascorbic acid.—

In frog stomach.—Small concentrationa (1 in 10^7) are inhibitory to A.C., potassium and slightly to tone. 1 in 10^6 decreases the response to A.C. and potassium, though the response to latter may also increase. 1 in 10^5–10^4 are depressant though 1 in 10^4 may slightly increase the response to A.C.

In dog stomach.—1 in 10^8 improves the response to A.C., potassium and tone. 1 in 10^7 has the opposite action though the response to potassium may increase. 1 in 10^6 increases the response to A.C. and decreases that to potassium; tone is decreased. Further concentrations (1 in 10^5–10^4) are depressant to A.C. and potassium, though the action on tone is variable. Small concentrations (1 in 10^8–10^7) may increase and large concentrations (1 in 10^5) may decrease tone.

Action of hesperidin.—

In frog stomach.—1 in 10^7 decreases the response to A.C. and potassium and increases tone. 1 in 10^6–10^5 decrease the response to A.C. but increase that to potassium and tone. 1 in 10^4 increases the response to A.C. and potassium and decreases tone.

In dog stomach.—1 in 10^7 increases the response to A.C., and decreases that to potassium, and greatly increases tone. The response to potassium may be oppositely affected. 1 in 10^6 increases the response to A.C., and tone now decreases when compared to that with 1 in 10^7. These actions increase with further increase in concentration (1 in 10^5). 1 in 10^4 decreases the response to A.C. and tone, and may increase that to potassium.

Discussion

The action of water-soluble vitamins follows the rule already laid down for the action of other substances. The excitability to A.C. and potassium may be similarly or oppositely affected, and tonic contraction is depressant to both.

PART III

Action of Anæsthetics

These and subsequent experiments were performed on frog stomach.

Action of ethyl alcohol.—1 in 10^7 increases the response to A.C., decreases that to potassium and does not affect the tone significantly. I in 10^6 increases the response to A.C. but to a lesser extent, and further decreases the response to potassium. 1 in 10^5 is more depressant to A.C. than 1 in 10^6, less depressant to potassium and decreases tone. 1 in 10^4 increases the response to A.C. and potassium but decresaes tone. 1 in 10^3–10^2 decreases the response to A.C. but increases that to potassium and tone; 1 in 10 has similar but more powerful action.

Action of methyl alcohol.—1 in 10^7 decreases tone and the response to A.C. and increases that to potassium. 1 in 10^6 has similar effect. 1 in 10^5 increases the response to A.C. and potassium and decreases tone; further concentrations (1 in 10^4–10^2) decrease the response to A.C. and tone and increase that to potassium. 1 in 10 is depressant all round; it does not cause tonic contraction.

Action of amyl alcohol.—1 in 10^7 is depressant to A.C. and potassium but increases tone. 1 in 10^6–10^4 is depressant to A.C., but increases the response to potassium and decreases tone. Higher concentrations are depressant.

Action of glycerol.—1 in 10^7 is depressant. 1 in 10^6 increases the response to A.C. and potassium but tone is diminished. 1 in 10^3–10^2 decreases the response to A.C., and increases that to potassium; tone increases and 1 in 10 causes a marked tonic contraction.

Action of chloroform.—1 in 10^7–10^6 increases the response to A.C., but diminishes that to potassium and tone. 1 in 10^5–10^4 decreases tone and the response to A.C., but increases that to potassium. Higher concentrations are depressant.

Action of ether.—Exact concentration was difficult to note, but as found previously (Singh, 1938), a certain concentration decreases the response to A.C., and increases that to potassium.

Action of chloral hydrate.—1 in 10^7 is inhibitory. 1 in 10^6–10^4 decreases the response to A.C. and increases that to potassium, 1 in 10^5–10^4 increasing tone as well. 1 in 10^3 decreases tone, so that the response to A.C. may again increase. 1 in 10^2–10^1 is depressant.

Action of procaine.—1 in 10^7 is inhibitory: 1 in 10^6 increases the response to A.C. and potassium and decreases tone. 1 in 10^5–10^4 decreases the response to A.C., and increases that to potassium and tone.

Action of cocaine.—1 in 10^7 increases the response to A.C. and decreases that to potassium and tone. 1 in 10^6 decreases the response to A.C. and tone and increases that to potassium. 1 in 10^5 is depressant. 1 in 10^4 causes tonic contraction.

Action of morphine.—1 in 10^7-10^6 increases the response to A.C. and decreases that to potassium; tone is not significantly affected. 1 in 10^5–10^4 decreases the response to A.C. and tone and increases that to potassium; 1 in 10^3 causes tonic contraction.

Discussion

Small concentrations of anæsthetics, as in the nervous system, may be inhibitory to unstriated muscle. The effect on the tone is variable depending upon the state of the muscle. Damaging concentrations increase the excitability to potassium and cause tonic contraction, and decrease the excitability otherwise.

PART IV

Action of Calcium Precipitants

Action of sodium sulphate.—1 in 10^7–10^6 increases as excitability to A.C. and tone. 1 in 10^5 increases the excitability to A.C. and potassium and tone. 1 in 10^4–10^2 increases the excitability to A.C., decreases that to potassium and increases tone. This increase in tone is not due to inactivation of calcium, as the latter has opposite effect, and the calcium affected would be much too small. 1 in 10 sodium sulphate increases the excitability to A.C. and decreases that to potassium and tone. High concentrations are depressant.

Action of sodium citrate.—1 in 10^7 increases the response to A.C. and potassium and decreases tone. 1 in 10^6–10^4 increases the response to A.C. and decreases that to potassium and tone. 1 in 10^3 has similar action, but may increase the response to potassium. 1 in 10^2 increases the response to A.C. and tone and decreases the response to potassium. Higher concentrations are depressant but cause tonic contraction.

Action of sodium fluoride.—1 in 10^7–10^5 increases the response to A.C., but decreases that to potassium and tone. 1 in 10^4 increases the response A.C. and potassium and tone. 1 in 10^3 decreases the response to A.C. and tone and decreases that to potassium. 1 in 10^2 is depressant, but 1 in 10 causes tonic contraction. With replacement of about 5% of the chloride of the saline with other halides, the excitability to A.C. increases in the following order:—$F < Cl < Br < I$.

Action of sodium oxalate.—1 in 10^7–10^5 increases the response to A.C. and decreases the response to potassium and tone. 1 in 10^4 increases the response to A.C. and potassium and tone. Higher concentrations are depressant.

Discussion

Substances which remove calcium from activity affect the excitability of unstriated muscle by diminishing adaptation and by producing other effects of calcium deficiency, which may be antagonistic. The former effect will increase the excitability and the latter effect will be variable. Besides the ions have no doubt their specifications.

PART IV

Action of Some Other Substances

Action of sodium acetate.—1 in 10^7–10^2 has no significant action. 1 in 10 increases the response to A.C. and tone and decreases that to potassium replacement of the chloride of the saline with acetate increases the response to A.C. and tone and decreases that to potassium.

Action of sodium cyanide.—1 in 10^7–10^6 is inhibitory. 1 in 10^5 increases the respone to A.C. and decreases that to potassium and tone. 1 in 10^4–10^2 has opposite action. Further increase in concentration of the cyanide causes a marked tonic contraction.

Action of atropine.—1 in 10^7 increases the response to A.C. and decreases that to potassium and tone. Further increase in concentration is depressant.

Action of quinine.—1 in 10^7 increases the response to A.C. and decreases that to potassium and tone. Further increase in concentration (1 in 10^6–10^3) is depressant to A.C. and potassium but increases tone.

Action of formaldehyde.—1 in 10^7–10^6 increases the response to A.C. and decreases that to potassium; tone is not significantly affected. 1 in 10^5 has the opposite action. 1 in 10^4–10^2 are depressant.

Discussion

There are following variables in the excitability complex of unstriated muscle, and these are affected independently by various agencies. (1) Excitability to electric current. (2) Excitability to potassium. (3) Tone. (4) Adaptation and inhibition. (5) Excitability to drugs. (6) Excitability to nervous stimulation. Viscosity may also be an independent factor.

It is possible that drugs act on the cell membrane, and thus altering its permeability, the action being specific like the opening of a lock by its key. If the permeability is decreased then the cell may be deprived of some vital substance, thus decreasing its activity. This may result in atrophy or even death as in the case of parasites. Increase in permeability by allowing increased access to raw materials required by the cell metabolism may have an opposite action. This might explain the action of minute quantities of drugs and hormones. It is possible that toxic doses might act in another way. If the permeability is too much increased, then toxic substances might diffuse in and so damage the cell or essential substances may diffuse out of the cell, and thus impair its activity. Thus if the permeability is increased potassium inside the cell may be replaced by sodium.

Bromides and iodides are known to cause eruptions in the skin of the human being. Thus anions cause unstriated muscle to swell. If the capillary walls of this particular part of the skin have special affinity for the bromides and iodides, they will swell and become more permeable and so the eruptions will ensue. If the permeability is increased, then sodium chloride would produce similar effects; other substances may produce such effects. As calcium prevents the swelling it is possible that the increase in permeability may result from release of calcium, and its subsequent diffusion away from the cells, or changes in permeability may be produced by changes in ionised calcium in the cells. The specificity of a substance may lie in its capacity to alter the calcium conceptration of the cell membranes. This suggestion is supported by the fact that the substances may exhibit specificity in causing inhibition of unstriated muscle; the latter is identical with adaptation or accommodation which is presumed to be due to changes in the free calcium in the cells. These will lead to changes in permeability, which in turn will affect the activity of the cell as presumed. This hypothesis gives a common explanation for the diverse physiological phenomenon such as the action of drugs on the unstriated muscle, the action of hormones, etc.

Substances that cause damage to the muscle, cause it to contract. These may be classed as irritants. What is the physiological nature of irritation? This appears to be an increase in permeability as irritants are known to cause hæmorrhages from capillaries.

In unstriated muscle, I have not been able to find any difference between fatigue and adaptation, and as the latter is identical with inhibition, it appears that in isolated muscle fatigue, inhibition, adaptation or accommodation are identical. This shows that even in isolated muscle, overactivity is prevented by concomitant inhibition as in the case of voluntary effort; in the former case inhibition is myogenic and in the latter case neurogenic.

Substances may cause contraction in small and relaxation in large concentrations (Singh, 1945). When excitation takes place in muscle it contracts, but the excitation subsides owing to adaptation or increase in inhibitory state. The muscle then relaxes, but then adaptation takes place to inhibitory state (adaptation to adaptation, or accommodation to accommodation) so that excitation recurs. This results in spontaneous or rhythmic contractions. This view is supported by the fact that in the presence of calcium most substances may not produce a contraction. If the calcium concentration is decreased, then spontaneous contrations occur, and if the calcium concentration is still further decreased, then tonic contractions occur; similar phenomena was found to occur in frog heart. Therefore fatigue, inhibition, adaptation, or accommodation and refractory period are identical, *i.e.*, all inhibitory states are the same. Any substance that causes contraction will cause relaxation under suitable conditions. Of these conditions, two can usually be successfully employed; one is to use a weak stimulus, and the other is to cause the muscle to contract, or diminish the excitability by means of a drug. The above are the methods which have been used to show the presence of vasodilatory fibres; it is clear that these methods are of doubtful utility.

Most substances affect the tone according to the state of muscle. If the muscle is sensitive to A.C., they may cause relaxation; if it is sensitive to potassium they may cause contraction. Former state can be produced by stimulation with A.C. and the latter with potassium. It appears that stimulation with A.C. results in the increase of the inhibitory state, so that when the muscle is stimulated by another agency, the inhibitory state predominates over the excitatory. This is probably due to the fact that all stimulants give rise to both excitatory, and inhibitory states, and if the threshold inhibitory state is already increased, then on addition of the stimulant, the combined inhibitory action of the stimulant and the resting inhi-

bitory state of the muscle exceed in action the excitatory action of the stimulant, *i.e.*, the same reason which accounts for the contraction of the relaxed muscle and relaxation, of a contracted muscle by a stimulant (Singh, 1945). The above is in agreement with the fact that *Mytilus* muscles which adapt rapidly, react by relaxation to most substances that usually cause contraction.

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