THE EFFECT OF CALOMEL ON PLASMA EPINEPHRINE IN THE RAT AND THE RELATIONSHIP TO MECHANISMS IN PINK DISEASE*

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Zahorsky (1922) stated that he was inclined to the view that calomel was the causative agent in pink disease. However, some patients were encountered having no history of calomel ingestion, so the idea was dismissed. Lesesne Smith of South Carolina considered that mercury was involved. His belief led Davison (1938) to include mercury as the aetiological agent in the 1938 edition of his current text. Ten years later scientific evidence for the involvement of mercury was furnished by the work of Warkany and Hubbard (1948, 1953) and by Fanconi and Botsztejn (1948).

The mode of action of mercury has not been understood, especially by workers investigating the autonomic dysfunction. They have tried to determine the nature of the dysfunction either by indirect physiological studies (Day, Smith and Klingman, 1939; Vulliamy, 1952; Bower, 1954; Farquhar, Crawford and Law, 1956) or by observation of the therapeutic effect of antisympathetic drugs (Gillespie, 1952; Bower, 1954; Peterson and Laughmiller, 1954). Their work supports the contention that excessive action of the sympathetic division exists. This contention is also supported by the findings that basal metabolic rate and venous pressure are increased in pink disease (Cheek, 1957). The methods involved in earlier attempts to measure blood epinephrine in this disease (Cheek, Hetzel and Hine, 1951) are now known to be inadequate.

Other work (Cheek and Hicks, 1950; Cheek, 1953) drew attention to the role of 'stress' in pink disease, to the failure of patients to withstand intercurrent infections, atmospheric heat, and intravenous hypotonic solutions, and of further electrolyte loss.

Preliminary work (Cheek and Hicks, 1950) indicated a state of salt loss. A significant reduction of the total body chloride and extracellular volume was demonstrated subsequently (Cheek, 1957). Since extracellular volume is intimately related to the production of aldosterone (Bartter, Liddle, Duncan, Barber and Delea, 1956), a state of functional adrenal insufficiency is thought to be present (Cheek, 1953). Urine 17-ketosteroid excretion is high and sweat sodium concentration is low, so that the production of steroids is adequate if not high. Since sweat water loss contains steroids and approximates or exceeds urine water loss, it seems impossible to measure accurately 11-oxysteroid excretion. A resistance to sodium-retaining steroids also has been demonstrated (Cheek, 1954) while the increase in red cell water (Cheek, 1957) may reflect a similar state of affairs in other tissue cells.

For the most part, all these electrolyte findings can be related to the actions of mercury. Calomel, the major offender, is a mercurial diuretic of bygone days. Mercurial diuretics produce an increase in cell hydration in rats, while calomel causes loss of body Cl and Na and retention of body K without change in renal structure (Cheek, Bondy and Johnson, 1959). Resistance to sodium-retaining steroids is compatible with the action of mercurial diuretics (Weston, Escher, Grossman and Leiter, 1952).

By contrast, the clinical features of hypertension, tachycardia, sweating and hypotonia do not appear, on the surface, to be related to the role of mercury. A study of the problem was undertaken by observing the effects of calomel and epinephrine on the young rat. It was found (Cheek *et al.*, 1959) that the ordinary effects of epinephrine (haemoconcentration, increased insensible water loss, hypertension, tachycardia) were perpetuated and intensified if the rat had received 10 mg. of calomel. Whether these

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results were due to an increased tissue response to epinephrine or to the maintenance of a high level of epinephrine in the rat is the subject of the present study.

Experimental Procedures and Methods

Male albino rats weighing 80 g. were divided into two equal groups, experimental and control. The experimental group received 10 mg. of calomel suspended in water by tragacanth, which was introduced into the stomach by a rubber catheter. Food and water were available, and the rats were placed in individual cages. After 22 hours the animals were lightly anaesthetized with ether and bled from the aorta, using a syringe containing 0.5 ml. of sodium thiosulphate-fluoride mixture to preserve the catecholamines, according to the technique of Weil-Malherbe and Bone (1952). In the second experiment the 'calomel' and control rats were placed in a refrigerated room (4°C.) with water but without food and left for 21 hours. Then they were anaesthetized and bled as before. In subsequent experiments 0.5 mg. of epinephrine in oil was injected intramuscularly into control and calomel rats. The calomel rats received their injection four hours after the administration of the mercurous chloride. Food and water was available. Twenty to 21 hours from the beginning of the experiment the animals were sacrificed as described. Before the injection of epinephrine in oil, the solution was taken from several ampoules and mixed thoroughly so as to ensure uniformity of dosage. The potency of epinephrine in oil varies slightly from ampoule to ampoule. The determination of epinephrine was carried out using the method described by Weil-Malherbe and Bone (1952) with the aid of a Coleman photofluorometer.

Results

In Table 1 the data for the plasma levels of epinephrine in the calomel and control rats are listed. Twenty-one hours after mercurous chloride adminis-

tration there was a slight increase in plasma epinephrine as compared with the control group. When the two groups of rats were subjected to stress from cold (4°C.), a fourfold increase in epinephrine level was seen in the calomel rats. In the subsequent experiments epinephrine in oil was injected 16 to 18 hours before sacrifice. Since epinephrine in oil was slowly liberated into the circulation, the results reflected the rate of destruction of the epinephrine concentration. Clearly, rats exposed to calomel had a concentration two or three times as high as control animals. These data from all experiments are statistically highly significant ($P < 0.01$).

Discussion

The present findings and those of the previous study (Cheek *et al.*, 1959) demonstrate the ability of calomel to augment the activity of the sympathetic branch of the autonomic nervous system. Stimulation of the sympathetic division by cold stress or by the direct injection of epinephrine gave rise to a higher level of plasma epinephrine if calomel was also present in the body. During the course of the present study a possible explanation for this phenomenon came to hand.

Axelrod and Tomchick (1958) found that epinephrine is destroyed in body tissues mainly by methyl transferase and not by mono-oxidase, as was believed previously. For this enzyme, methyl transferase, to work, a substrate adenosyl methionine is necessary. The latter substrate contains sulphhydryl groups and is inhibited by inorganic mercury.

Calomel is the source of mercury in pink disease in most instances (calomel disease), and it seems reasonable to believe that the same potentiation of sympathetic activity can occur in the clinical situation.

Evidence has accumulated that the formation of haem from iron and protoporphyrin is enzyme-dependent (Schwartz, Cartwright, Smith and Wintrobe, 1959). The enzyme requires sulphhydryl groups for its action, and it is possible that the high reticulocyte count noted in some patients with pink disease, and the slightly increased porphyrin excretion in the urine (Cheek *et al.*, 1951) are due to the fact that mercury inhibits sulphhydryl groups.

From the present data and from previous work it is possible to suggest the mechanisms which are active in pink disease, and to explain the role of mercury (Fig. 1).

If activity of the sympathetic branch of the autonomic nervous system is enhanced in the infant by previous illness or by environmental or other factors, the response of the sympathetic division will tend to

TABLE 1
PLASMA EPINEPHRINE LEVELS ($\mu\text{g./l.}$)

Experiment	No. of Rats per Group	Remarks	Control Rats +S.D.	Calomel Rats +S.D.
1	7	22 hr. after 10 mg. calomel	2.15 ± 0.33	4.01 ± 0.54
2	5	20-21 hr. after calomel; stress of 4°C.	2.34 ± 0.21	9.18 ± 0.72
3	5	20-21 hr. after calomel; 16 hr. after 0.5 mg. epinephrine	14.89 ± 1.55	31.72 ± 5.10
4	8	20-21 hr. after calomel; 16 hr. after 0.5 mg. epinephrine	8.37 ± 1.60	23.20 ± 3.60
5	6	20-21 hr. after calomel; 18 hr. after 0.5 mg. epinephrine	2.60 ± 0.20	7.04 ± 1.47

S.D. = standard deviation.

FIG. 1.—Suggested mechanisms in pink disease.

be perpetuated if calomel or another inorganic mercurial is present. Calomel can also act as a mercurial diuretic and can reduce body NaCl and extracellular volume, which in turn would produce endogenous sympathetic activity.

In earlier years, when a high salt intake and desoxycorticosterone was used experimentally, a dramatic improvement was observed in some patients but not in others (Cheek and Hicks, 1950; Murray, 1950; Cheek *et al.*, 1951; Forman, 1951; Williams, MacDonald and Callow, 1951). It is possible that some patients do respond to steroid with expansion of extracellular volume, a result which could break a vicious cycle (Fig. 1).

Stimulation of the sympathetic branch of the autonomic nervous system by the injection of epinephrine produced damage to the cortico-medullary junction of the rat kidney if calomel was also present (Cheek *et al.*, 1959). Renal pressor factors may also be important in pink disease in the maintenance of vasospasm.

The sympathetic division of the autonomic nervous system seems to be more active in infants than in adults. Perhaps this accounts for the clearly defined age incidence of the disease. In earlier years it was suggested that functional adrenal

cortical insufficiency was present in pink disease, and to this we can now add maximal activity of the adrenal medulla. It is agreed generally by adrenal physiologists that if sympathetic activity overrides adrenal cortical activity there is a failure of adaptation or failure to meet added stresses (Ramey and Goldstein, 1957). It seems that the nature of pink disease includes a predominating adrenal medullary action together with a functional cortical insufficiency, all of which is related to the presence of inorganic mercury in the body. This disease should find a place adjacent to pheochromocytoma in the current texts.

Removal of as much mercury as possible from the body seems important, together with the administration of anti-sympathetic drugs and efforts to restore extracellular electrolyte. It may also be important to supply additional sulphhydryl groups in the form of methionine.

Summary

Previous experiments on young rats, by indirect methods, demonstrated that calomel potentiates the effect of epinephrine on the body. The present study presents direct data on the plasma epinephrine concentration. Rats subjected previously to a small

dose of calomel had higher plasma epinephrine concentrations than control animals. The difference in plasma epinephrine concentrations would be magnified by subjecting the animals to cold stress, or by the injection of 0.5 mg. of epinephrine in oil.

It is suggested that in the clinical counterpart, pink disease or calomel disease, there is the same potentiation of sympathetic activity. In the light of the present and previous work, the mechanisms operating in pink disease are suggested.

Attention is drawn to the possibility that stress or a previously activated sympathetic system may be aetiologically important in pink disease.

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