17.7%. The changes in CBF produced by KCl infusion did not parallel the changes in plasma K concentration. Infusion of 2,4-dinitrophenol or epinephrine, asphyxia, or increased aortic pressure (all factors which are known to increase myocardial oxygen consumption and coronary blood flow) did not result in the release of K from the myocardium. Therefore, it appears unlikely that K release from active myocardium is responsible for adjustment in coronary resistance which accompanies changes in metabolic activity of the myocardium.

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Received September 9, 1957. P.S.E.B.M., 1957, v96.

Reactivity of Sulfhydryl and Disulfide Groups in Serum of Man.* (23522)

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Sulfhydryl-containing compounds such as cysteine and cysteinamine have been shown to be very effective in protecting against the lethal effects of ionizing radiations(1-3). As one approach to understanding the mechanism underlying this protection we have measured in human serum the levels of sulfhydryl (–SH) and disulfide (–S–S–) and their reaction to ultraviolet (UV) light, detergent, and electrolysis.

Materials and methods. The serum used in these studies was freshly prepared from the blood of young (20-25 years) and older (40-50 years) human subjects and from various types (metastatic carcinoma of the throat and rectum) of cancer patients from the Ghent University Clinic. The sulfhydryl and disulfide determinations were accomplished using one of the amperometric methods described by Kolthoff *et al.*(4). The –SH determination was carried out in an ammonia buffer (0.1 M NH_4OH, 0.3 M NH_4NO_3); after deaeration of the buffer solution with purified nitrogen(5) the serum was added and titrated with a 0.002 or 0.001 M solution of $AgNO_3$ at –0.30 volt, *vs.* a standard calomel electrode. The total volume was 20 ml. The –S–S– determination was carried out in ammoniacal medium in the presence of sulfite (0.1 M NH_4OH, 0.1 M NH_4NO_3, 0.2 M Na_2SO_3). The $AgNO_3$ solution and the potential were identical to that used in the –SH determination. The nitrogen content of the serum was determined by an amperometric method which makes it possible to analyze very minute quantities of serum (down to 0.02 ml) in 15 minutes(6). UV irradiation. A UV lamp (type 16200, Hanovia Chemical and Manufacturing Co., Newark, New Jersey) was used to irradiate a quartz cell with a diameter of 1.27 cm and a length of 8.40 cm. The distance from the cell to the lamp was 6 cm. An aliquot of serum, diluted 1:16 with air-free phosphate buffer (0.1 M Na_2HPO_4, 0.01 M NaH_2PO_4; pH 7.29), was pipetted into the cell, which was closed hermetically after purified nitrogen had been passed over the sample(4). A current of cold air was blown over the cell in order to avoid a rise of temperature during the experiment; after 2 hours of irradiation the temperature rose 1°C. Aluminum foil was used as a reflector behind the cell. Anionic Detergent. To test the effect of an anionic detergent upon –SH and –S–S– groups, 0.5 ml of serum was placed in 20 ml

* This work performed under the auspices of the U. S. Atomic Energy Com. under the Belgian-American Agreement Concerning Atomic Energy.

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of 7.5 x 10^{-5} M sodium laurylsulfate for 15 minutes. The –SH and –S–S– determinations were carried out according to the technic mentioned above. Electrolysis. Different methods have been used in order to reduce –S–S– in proteins. One of these is reduction with sodium amalgam (10%) in acid or basic medium. Using this method we found that after 30 minutes the –SH value of serum rose from the normal 6.7 mg/100 ml only to 8.7 mg/100 ml, which was not adequate for our purposes. Hata(7) has described a technic for oxidizing cysteine to cystine in different acid media by electrolysis. By applying a modification of this technic to serum proteins we were able to obtain a high degree of reduction of –S–S–. Serum was used in a 1:20 dilution in a medium containing 0.05 M HCl and 0.5 M KCl. The applied potential was 90 volts, over an agar bridge saturated with KCl; the current was 40 ma. The reaction was carried out in an air-free medium(4), and the thermostat was set at 25.00° C ± 0.02.

Results. After 2 hours of UV irradiation the –SH content of a sample of normal serum was 42 mg/100 ml. When the proteins of the serum were precipitated with 20% sulfosalicylic acid, the filtrate contained only 1.3 mg –SH, which indicates that the –SH of the serum is still contained in proteins of high molecular weight.

To investigate the behavior of –S–S– groups under influence of UV light, 4 ml of 5 x 10^{-5} M cystine solution in 0.05 M HCl was placed in a quartz cell and diluted to 8 ml with air-free phosphate buffer. After irradiation of the solution for 30 minutes, 0.724 x 10^{-5} moles –S–S– was reduced to –SH, 2.07 x 10^{-5} moles of –S–S– was unchanged, and 44% of –S–S– was lost by oxidation or breakdown of the molecule. After UV irradiation of a 7% albumin solution diluted 1:16 with the air-free phosphate buffer, the content of –SH increases with the duration of irradiation (Fig. 1). In the case of normal serum a greater amount of –SH and –S–S– is found after irradiation. We shall refer to this increase in detectable –SH and –S–S– as reactivity. Fig. 2 gives an example of normal serum titrated after different periods of irradiation. During these experiments it was also found that non-irradiated serum contains reactive –S–S– groups, which are amperometrically detectable. When one-day-old serum was used, the –SH and –S–S– contents were lower than that of fresh serum, and the reactivity of the groups was lower.

In cases of cancerous serum the same phenomenon was manifested. However, the reactivity is much greater; there are more titratable –SH and –S–S– groups after the irradiation period, compared with normal serum, and fewer –SH and –S–S– groups detectable in the untreated serum (Fig. 3).

In young persons up to 25 years the average –SH content of the serum is 6.8 mg/100 ml serum (mean of 200 individuals, range 5.6 to 8.6 mg/100 ml serum). In older persons (40 years of age) the average content is 5 mg/100 ml serum (mean of 200 individuals, range 4.3 to 6.9 mg/100 ml serum). The reactivity of the –SH groups after UV radiation is, however, much greater in older persons than in the case of younger ones.

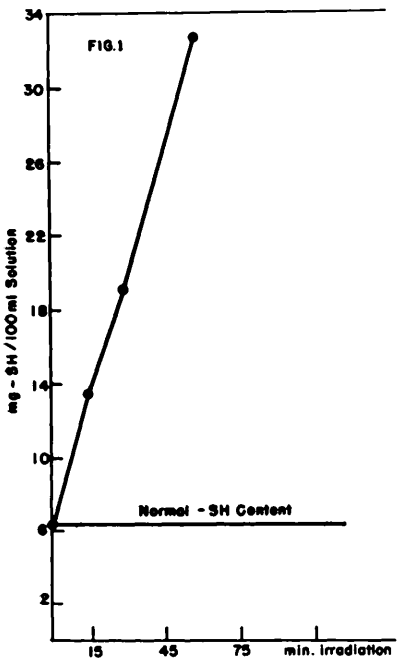
The average –SH content of cancerous serum is 3.3 mg –SH/100 ml serum (mean of 150 individuals, range 2.3 to 5.4 mg/100 ml serum), the reactivity of the groups is much higher compared with the sera of normal persons. The –S–S– content averages 7.2 mg/100 ml serum (range 4.0 to 9.2 mg/100 ml serum) for normal persons, and 12.1 mg/100 ml serum (range 7.3 to 14.0 mg/100 ml serum) in the cases of cancerous serum. The overlapping of the –SH and –S–S– contents between normal and cancerous sera makes a differentiation on that basis difficult; the error factor is 12%.

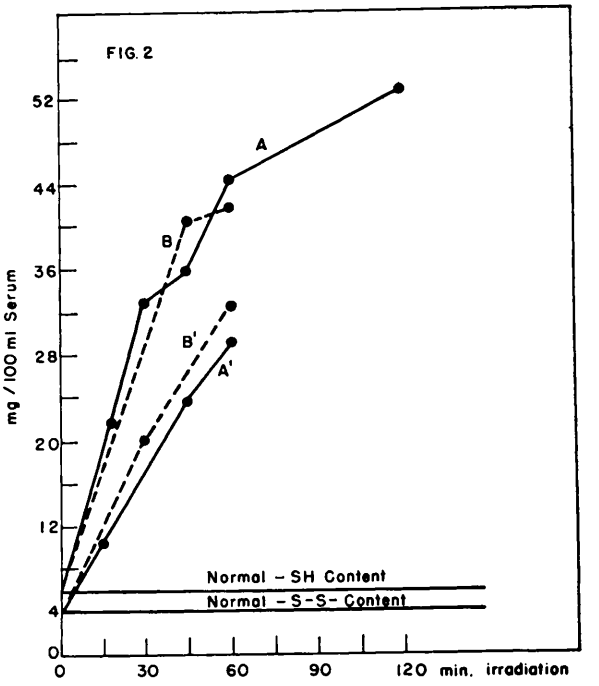
The greater amount of –SH and –S–S– found after irradiation can be explained by the unfolding property of the molecules, by which more of these groups become free, a reaction that occurs also when the molecules are under influence of a detergent.

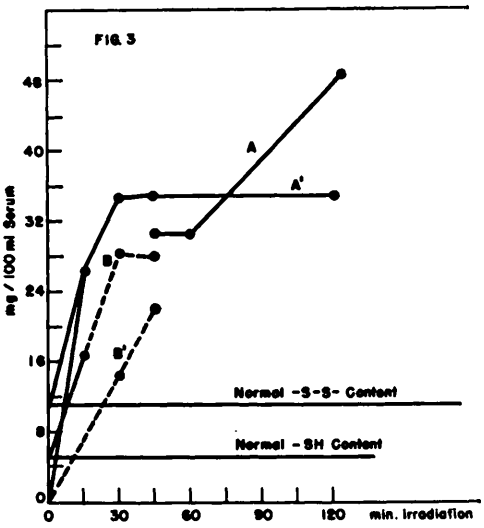
Cystine and serum behave differently under the influence of UV light; cystine is destroyed, while serum gives more titratable –SH and –S–S– groups.

The reactivity of these two groups is of great significance if correlated with the work of Bacq *et al.* (1) and Maisin *et al.*(2) who studied the protective properties of sulfhy-

dryl compounds such as cysteinamine (β-mercaptoethylamine, Bécaptan). The effectiveness of this compound can perhaps be explained by absorption of radiation energy, as shown previously in the case of cysteine. Another, and more likely possibility(8) is that complexes are formed between the compound and the other –SH and –S–S– groups in the







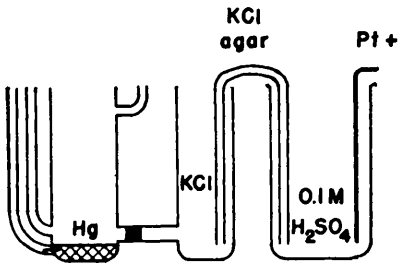


FIG. 4 ■ Sintered glass plate electrolysis cell

FIG. 1. Amperometric –SH titration of a 7% albumin solution after UV irradiation.

FIG. 2. Amperometric –SH and –S–S– determination of normal serum after UV irradiation; dilution 1 : 16. A, normal –SH content of fresh serum; A′, normal –S–S– content of fresh serum; B, normal –SH content of serum one day old; B′. normal –S–S– content of serum one day old.

Fig. 3. Amperometric –SH and –S–S– determination of cancerous serum after UV irradiation; dilution 1 : 16. A, normal –SH content of fresh serum; A′, normal –S–S– content of fresh serum; B, normal –SH content of serum one day old; B′, normal –S–S– content of serum one day old.

FIG. 4. No text.

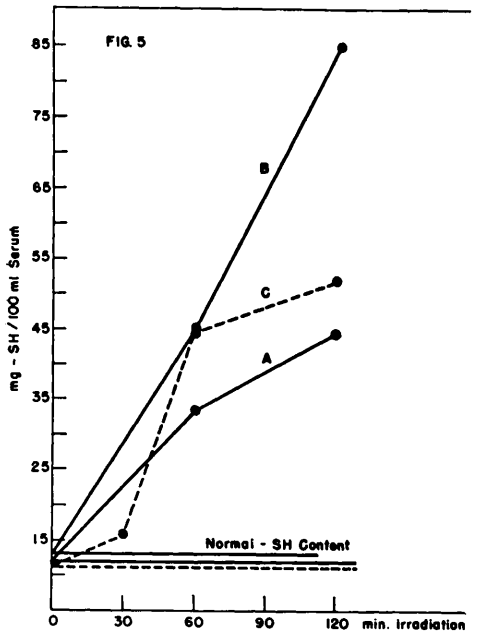


FIG. 5. Amperometric –SH titration of normal serum (A and C) and a 7% albumin solution (B) after electrolysis; 1 ml in 0.05 M HCl and 0.5 M KCl. Total volume 20 ml, current 40 ma, 90 volts.

serum. This complex formation can be shown polarographically; the stoichiometric equivalent could not be determined. In this reaction the amino groups seem to play an important role.

Under the influence of a detergent, sodium laurylsulfate, an effect similar to that of UV light was noticed. However, the amounts of –SH and –S–S– released were lower, and the difference between sera of young and older persons was also less distinct.

If the serum proteins were precipitated in pH 3.7 acetate buffer (0.1 N acetic acid, 0.1 N sodium acetate) and redissolved in pH 9 ammonia buffer, the –SH and –S–S– values were higher than those obtained by direct determinations under the influence of the detergent without any precipitation. These experiments make it clear that under the influence of a detergent the spiral structure of the proteins unfolds, and a greater number of –SH and –S–S– groups can be detected.

After 4 hours of electrolysis (Fig. 4) 92 mg of –SH per 100 ml of a normal serum were detected (Fig. 5). The reaction of a 7% albumin solution is given for comparison. The results of these experiments show again that sera of younger persons have a lower reactivity compared to those of older persons. However, as in the experiments with sodium laurylsulfate, the difference is less marked than in the experiments with UV.

After electrolysis in basic medium (0.5 M NH_4OH and 0.25 M NH_4Cl), no greater amounts of –SH and –S–S– could be detected; *e.g.*, after 2 hours no –SH could be determined and a cathodic current appeared. The nature of this reaction has not been determined.

Summary. 1) Reactivity of sulfhydryl and disulfide groups in normal and cancerous sera was studied under the influence of UV radiation, detergents, and electrolysis. We were able to demonstrate that serum as such contains reactive –S–S– groups, and that the reactivity of –SH and –S–S– differs according to age and for normal and cancerous serum. 2) The greater amount of –SH and –S–S– found after irradiation could be due to unfolding of the spiral structure of the protein and consequent release of the residual bonds. The energy required for normal serum is greater in the case of younger people (up to 25 years) than for older people (aging effect) and also greater for normal serum than for cancerous ones. 3) Sodium laurylsulfate also greatly increased the –SH and –S–S– content, which can be explained by the unfolding property of the molecule. The electrolysis of serum in acid medium made it possible to reduce –S–S– groups in proteins; the response of serum to this treatment is described. The possible relation of these results to the protective action of –SH against irradiation is discussed.

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Received September 9, 1957. P.S.E.B.M., 1957, v96.

Humoral Conditioning for Production of Acute, Massive Myocardial Necroses by Neuromuscular Exertion.* (23523)

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Previous experiments have shown that 2-methyl-9(α)-chlorocortisol (Me-Cl-COL), administered in combination with either basic or acid phosphates, results in acute, massive myocardial necroses in the rat(1). The possible relationships between this experimental lesion and the cardiac infarcts of man have not yet been studied. Since it is well known that neuromuscular effort is likely to precipitate myocardial infarction in predisposed people, the question arose whether this would also be the case in rats sensitized by threshold amounts of Me-Cl-COL and phosphate.

Materials and methods. Eighty female Sprague-Dawley rats, with an average initial body-weight of 101 g (range: 95-110 g), were subdivided into 4 equal groups, as indicated in Table I. Me-Cl-COL† was administered subcutaneously, in the form of its acetate, as a microcrystal suspension of 50 μg in 0.2 ml of water, once daily. NaH_2PO_4 was given by stomach tube, as a 15% aqueous solution, at the dose of 2 ml, twice daily. Neuromuscular strain was induced by maintaining the rats on a board with adhesive tape, for a period of 17 hours, starting 78 hours after the initiation of the Me-Cl-COL and NaH_2PO_4 treatment. The animals of all 4 groups were killed with chloroform, 5 hours after the release of the restrained rats. The hearts and the kidneys of half of the animals in each group were fixed in Susa solution for subsequent staining with hematoxylin-eosin, and those of the other half in neutral formalin for the subsequent demonstration of calcium deposits by v Kossa's silver nitrate stain. For both of these purposes the tissues were embedded in paraffin.

Results. The principal results of these experiments are summarized in Table I.

Upon naked-eye inspection at autopsy, the most striking change was the presence of large, light-yellow patches throughout the myocardium in all the animals of Group IV (Fig. 1). Similar, though less extensive lesions were also seen in 20% of the rats in Groups II and III, but not in Group I.

TABLE I. Humoral Conditioning for Production of Acute, Massive Myocardial Necroses by Neuromuscular Exertion.

Group	Treatment	Myocardial lesions Incidence	Myocardial lesions Severity*	Nephrocalcinosis Incidence	Nephrocalcinosis Severity*	Mortality (%)
I	Me-Cl-COL	0	0	0	0	0
II	" + Restraint	20	.3	0	0	0
III	" + NaH_2PO_4	20	.4	100	2.0	0
IV	" + Restraint + NaH_2PO_4	100	2.6	100	2.0	50

* Severity of the lesions was assessed both macro- and microscopically, in terms of an arbitrary scale of 0-3; figures in this column are the means of these readings.

* Supported in part by a grant from the Gustavus and Louise Pfeiffer Research Foundation and by a Grant from the Muscular Dystrophy Association of Canada.

† Generously supplied by The Upjohn Co.