that the main column of the α -track must play an important part.

Bonet-Maury & Lefort (1948) have shown that hydrogen peroxide is formed in pure water by α -radiation, and therefore part of the α -radiation effect could be due to the presence of hydrogen peroxide if the solute is sensitive to it. The presence of a solute however may reduce the formation of hydrogen peroxide by reacting with the primary products of radiations (OH radicals and H atoms). Carboxypeptidase was shown to be insensitive to high concentrations of hydrogen peroxide (Dale, Gray & Meredith, 1949) and though the hydrogen peroxide can deaminate glycine, the effect is not more than 12% of the total radiation effect as shown by the present control experiments.

Since neither the quality of the radiation nor the effect of hydrogen peroxide can wholly account for the observed differences, one should assume that these are linked in some way with the fact that the X-radiation effects on amino-acids were very different from those on macromolecules, as was fully discussed in an earlier paper (Dale, Davies & Gilbert, 1949). There a chain reaction or the action of radicals by excitation was invoked for explaining the kinetics of

the deamination of amino-acids by X-rays, and it was also pointed out that these theoretical considerations are not yet fully in agreement with current concepts of the indirect action of radiation.

SUMMARY

1. The deamination of glycine by the densely ionizing particles from the disintegration of boron by slow neutrons in a nuclear reactor has been studied. An account of the necessary technique is given and the dosimetry fully discussed.

2. The ionic yields for the liberation of ammonia were 0.17 and 0.56 for 1 and 20% solutions of glycine, respectively, being 15 and 19% of that due to X-radiation. The higher efficiency of deamination by this radiation compared with the inactivation of an enzyme by α -rays from radon and its products is discussed.

We wish to thank the Director, Atomic Energy Research Establishment, Harwell, for permission to make the irradiation in the nuclear reactor and the staff of the Isotope Division for information concerning the neutron flux, for a standardized solution of ^{42}K and for the assistance and facilities given during the irradiation.

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Observations on Antisera Against Complexes of Di-2-chloroethyl sulphide and Protein

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Human beings and animals, after receiving repeated skin exposures to di-2-chloroethyl sulphide (mustard gas, 'H'), often develop an increased sensitivity to this substance, and it was felt that the factors concerned might be investigated by methods developed in the study of immunity. Berenblum & Wornall (1939) previously published an account of an investigation undertaken with this end in view, and the

present communication provides additional information about the system first described by these authors. It was also hoped that by studying different derivatives of diethyl sulphide as inhibitors in a specifically precipitating immunity system, some evidence might be obtained for the nature of the linkage formed when H reacts with proteins.

There have appeared in recent years a number of reports describing the action of *H* on proteins, e.g. Banks, Bournsnel, Francis, Hopwood & Wormal (1946); Bournsnel, Francis & Wormal (1946*b*); Herriott, Anson & Northrop (1946); and Ogston (1948). The serological investigation which forms the subject of the present communication has disclosed another factor in such reactions, viz. the participation of inorganic phosphate.

EXPERIMENTAL

Preparation of compounds

Di-2-(2'-amino-2'-carboxyethylthioethyl) sulphide. Prepared by interaction of *H* with cysteine in the manner described by Hellerman (1942), this had m.p. 245°.*

1:4-Thiazan-4-acetic acid and 2-hydroxy-2'-glycino-diethyl sulphide. Ethyl aminoacetate and *H* were brought into reaction in absolute ethanol in the presence of anhydrous sodium acetate, and the crude oily product was isolated following the method of Cashmore & McCombie (1923). Two fractions were obtained from this product, one with b.p. 91–92°/1 mm., the other with b.p. 100–140°/1 mm. Each of these fractions was dissolved in dry ether, dry HCl gas was bubbled through the solution and the crystalline hydrochloride which separated in each case was collected on the filter with suction and recrystallized from an ethanol ether mixture (2/5).

From 10 g. of the oil (b.p. 91–92°/1 mm.) 10 g. of recrystallized hydrochloride with m.p. 185–187° were obtained. (Found: C, 42.5; H, 7.1; N, 6.2; S, 14.4; Cl, 15.7%. The hydrochloride of ethyl 1:4-thiazan-4-acetate, $C_8H_{16}O_2NClS$, requires C, 42.6; H, 7.2; N, 6.2; S, 14.2; Cl, 15.8%.) This ethyl ester hydrochloride (5 g.) and *n*-NaOH (100 ml.) were refluxed for 4 hr., after which the mixture was cooled; *n*-H₂SO₄ (78 ml.) was then added and the solution distilled to dryness *in vacuo*. The residue was extracted three times with boiling ethanol, the extracts filtered and reduced to dryness, and the residue crystallized from ethanol. Yield, 3.0 g. The resulting needle-shaped crystals showed a peculiar behaviour in melting-point determinations. In a capillary tube they melted at 130°; above this temperature some samples decomposed with much evolution of gas, while other samples recrystallized and melted at 175°. On the heated stage of a Fisher-Johns micro melting-point apparatus they had m.p. 175–176° with no preliminary melting at 130°. This transient melting at 130° was probably due to loss of water of crystallization, since analyses showed that the crystals contained water. A sample of the crystals was sublimed at 160°/0.15 mm.; the sublimate melted and turned red at 177–179° without any preliminary melting at 130°. (Found for the sublimate: C, 44.5; H, 7.4; N, 8.5; S, 20.2. Calc. for $C_8H_{11}O_2NS$: C, 44.7; H, 6.9; N, 8.7; S, 19.9%.) There seems little doubt that this material is identical with the 1:4-thiazan-4-acetic acid described by Bournsnel, Francis & Wormal (1946*a*).

From 7 g. of the oil (b.p. 100–140°/1 mm.) 2 g. of recrystallized hydrochloride with m.p. 120–122° were obtained. (Found: C, 42.0; H, 6.9; N, 5.1; S, 11.4; Cl, 12.1. Calc. for the ethyl ester hydrochloride of 2-acetoxy-2'-

glycino-diethyl sulphide, $C_{10}H_{20}O_4NClS$: C, 42.1; H, 7.05; N, 4.9; S, 11.4; Cl, 12.4%.) This ethyl ester hydrochloride (2 g.) and *n*-NaOH (30 ml.) were refluxed for 2 hr., after which the mixture was cooled, *n*-H₂SO₄ (20 ml.) was added and the solution was distilled to dryness *in vacuo*. The residue was extracted three times with boiling ethanol, the extracts filtered and reduced to dryness, and the residue crystallized by dissolving it in 10 ml. of hot ethanol, adding 2 ml. of acetone and chilling. The crystals separated as rosettes of needles melting at 129–131°. The material was very soluble in water, ethanol and methanol, but relatively insoluble in acetone, ether and benzene. (Found: C, 40.3; H, 7.4; N, 7.8; S, 18.0. Calc. for $C_8H_{13}O_3NS$: C, 40.2; H, 7.3; N, 7.8; S, 17.9%.)

The analyses of this material and of the ester hydrochloride from which it was derived provide good, though not conclusive, evidence that it is 2-hydroxy-2'-glycinodiethyl sulphide, and it is quite conceivable that the acetate of the ethyl ester of this compound could have arisen as a result of the reaction of 1 mol. of *H* simultaneously with 1 mol. of sodium acetate and 1 mol. of ethyl aminoacetate.

In the course of several preparations of the sort just described many attempts were made to obtain the di-2-glycinoethyl sulphide described by Cashmore & McCombie (1923), but without success.

H-glycine.† Glycine (10 g.), water (200 ml.) and *H* (30 ml.) were placed in a 500 ml. beaker and stirred continuously at 37°, 2*N*-NaOH being added at a rate sufficient to keep the reaction alkaline to phenolphthalein. After 8 hr. the consumption of alkali had ceased when 260 ml. of 2*N*-NaOH had been added. The reaction mixture was then extracted three times with chloroform and the chloroform washings discarded. The aqueous solution was concentrated *in vacuo* on the steam bath to about 100 ml. when a large amount of NaCl had separated; the sludge in the distilling flask was filtered with suction and the solid on the filter washed with a little cold water. The pH of the filtrate was adjusted to about 2 by the addition of conc. HCl. As the acidification proceeded a copious, gummy, slightly yellow precipitate formed and rapidly settled to a gummy mass at the bottom of the flask. After the mixture had been chilled for 2 hr. at 5° the supernatant liquid was decanted and discarded and the gummy precipitate washed twice with cold water. The remaining gum was then dissolved in 100 ml. water by adding 2*N*-NaOH until the pH of the solution was 6.5. The precipitation with acid, washing and redissolving of the product was carried out twice more; the resulting gum was dissolved in water at pH 6.5 and the solution diluted to 50 ml. This solution had 5.3 mg. N/ml.; yield, 14% (based on N).

A solution which had been made from a similarly prepared and purified reaction product gave: total N (Kjeldahl) 0.93 mg./ml.; amino N (Van Slyke), 0.00 mg./ml.; S, 1.46 mg./ml. When 5 ml. of this solution was mixed with 0.1 ml. of a 10% solution of any one of cobaltous acetate, cadmium chloride, nickel nitrate or cupric sulphate a copious precipitate formed. The chemical properties of this *H*-glycine received considerable study which will be the

† The '*H*-glycine' referred to here is not to be confused with the '*H*-glycine' used by Bournsnel *et al.* (1946*a*) to mean 1:4-thiazan-4-acetic acid, but refers to the unidentified product of the reaction of *H* and glycine in aqueous solution.

* All m.p. are uncorrected.

subject of a communication by Drs D. E. Douglas and R. D. H. Heard.

'Thiodiglycol phosphate.' $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ (6.7 g.) was dissolved in water (100 ml.) and the solution was maintained at 37° ; 4 g. of H (m.p. 14°) were then added and the reaction mixture stirred continually. The reaction was kept just alkaline to phenolphthalein by the addition of 2N-NaOH. After 2 hr., when the reaction was over, 19 ml. of 2N-NaOH had been used and an analysis for inorganic

On electrometric titration of the barium salt of 'thiodiglycol phosphate' and sodium phenyl phosphate it was found that the former bound more alkali between pH 9 and 12.2 than did the latter. The extra alkali bound was approximately one equivalent for three atoms of phosphorus.

In view of the above findings and the fact that H in aqueous solution readily gives rise to sulphonium salts (Herriott, 1946), the structure proposed for the barium salt of 'thiodiglycol phosphate' is

phosphate showed that 80% of that originally present had been esterified. The reaction mixture was distilled to dryness *in vacuo* and the residue was washed with absolute ethanol, the washings being discarded. The residue was then dissolved in 5 ml. of water, 50 ml. of absolute ethanol were added and the mixture was chilled at 5° for 3 hr. when the clear supernatant liquid was decanted and discarded. The solution and precipitation were carried out twice more after which the residue was dissolved in water and diluted to 50 ml. This solution gave: P (total), 12.5 mg./ml.; P (inorganic), 3.6 mg./ml.; S, 8.2 mg./ml.

The above solution was made alkaline to phenolphthalein by the dropwise addition of a saturated $\text{Ba}(\text{OH})_2$ solution; the precipitate which formed was centrifuged and discarded. To the supernatant fluid $\text{Ba}(\text{OH})_2$ dissolved in hot water was added in the proportions of one atom of Ba for each atom of P. The precipitate which resulted was centrifuged and washed three times with small volumes of distilled water. To the combined supernatant and washings, which were perfectly clear and colourless, an equal volume of ethanol was added and the resulting voluminous precipitate centrifuged, washed twice with 50% and twice with 95% ethanol, and dried *in vacuo* over CaCl_2 . (Found: C, 10.0; H, 2.8; S, 6.3; P, 9.5; Ba, 41.2. $\text{C}_8\text{H}_{16}\text{O}_{13}\text{S}_2\text{P}_3\text{Ba}_3 \cdot 5\text{H}_2\text{O}$ requires: C, 9.8; H, 2.7; S, 6.6; P, 9.5; Ba, 42.1%.)

It can be seen that the ratio P/S in this material is 3:2 and at one time it was suspected that this might be due to a mixture of thiodiglycol mono- and di-phosphates. In order to test this possibility a brucine salt was prepared and analysed for P and S. Aqueous solutions of the above Ba salt (0.5 g.) and brucine sulphate (1.5 g.) were mixed, BaSO_4 was removed by centrifuging and the resulting solution was evaporated *in vacuo*. Crystallization of the residue from water yielded 0.663 g. of fine white needles. Even after recrystallization this brucine salt had no definite m.p. but softened, melted and decomposed gradually from 110 – 185° . (Found: S, 2.32; P, 3.35%.) These analytical results indicate that the Ba salt was a single compound.

On drying the Ba salt in the pistol *in vacuo* over P_2O_5 it lost weight, but quantitative water determinations were impossible. The weight loss increased steadily as the temperature in the pistol was raised from 100 to 190° , until at the latter temperature the material turned brown and was obviously decomposing.

If the substance contains a sulphonium salt grouping it should be in the form of the sulphonium hydroxide when it separates from the alkaline baryta solution. The slightly high carbon content found on analysis might be due to a partial neutralization of the sulphonium hydroxide with CO_2 . It seems impossible to reconcile the analytical results with any other reasonable structure, but unfortunately the proposed constitution does not readily lend itself to proof by degradation or synthesis.

For use in specific inhibition tests the Ba salt was dissolved in water and Ba removed by the careful addition of 1% Na_2SO_4 solution to slight excess. The resulting solution, after removal of BaSO_4 and adjustment of volume, was used as an inhibitor.

Reaction of H with serum proteins. Preparations were made from the serum proteins of the horse, rabbit, guinea pig, pig and man.

(a) *H-horse serum globulin (phosphate, pH 8-9).* Horse serum globulin solution (50 ml., containing 0.46 g. N) was mixed with 0.5M-phosphate buffer (25 ml., pH 8) and warmed to 37° . H (3 ml.) was added and the mixture stirred constantly and maintained at 37° . 2N-NaOH solution was added at such a rate as to keep the mixture just alkaline to phenolphthalein. After 8 hr. the reaction was finished and 19 ml. of 2N-NaOH solution had been used. The resulting solution was placed in a cellophane sac and dialysed against running tap water for 48 hr. The contents of the sac were clarified by filtering, and analyses for N and S were carried out on the solution. (At first, samples of the solution of antigen were precipitated with acetone and the precipitate, after having been washed, and dried *in vacuo*, was analysed for S and N. Since it was later found that the same values for S/N were obtained when the analyses were carried out on samples of solution, the acetone precipitation procedure was discontinued.) In each case, analyses for S and N were carried out on the original protein before treatment with H . Analyses for the above coupling were: original horse serum globulin solution: S/N, 0.09; coupled horse serum globulin solution: S/N, 0.34; Δ (S/N), 0.25. The final solution was stored at 5° with a few crystals of thymol as a preservative.

(b) *H-horse serum albumin (phosphate, pH 8-9).* Horse serum albumin solution (75 ml., 8.5 mg. N/ml.), 0.5M-phosphate buffer (33 ml., pH 8.0) and H (4 ml.) were

stirred at 37°. 2N-NaOH (27.5 ml.) was added at a rate sufficient to keep the reaction just alkaline to phenolphthalein. After 4 hr. the reaction was finished and the resulting mixture was dialysed in a cellophan sac against running tap water for 4 days. The contents of the sac were then filtered and the filtrate electro dialysed against running distilled water until no current flowed through the cell. Early in the dialysis, a gelatinous precipitate began to form and this increased in amount throughout the operation. The dialysed mixture was centrifuged and the supernatant fluid (215 ml.) had a pH of 4.5 and contained 0.07 mg. N/ml. and 0.000 mg. total P/ml.; it was discarded. The gelatinous precipitate was washed once with distilled water, centrifuged and the washing discarded. The precipitate was treated with 150 ml. water and enough N-NaOH to bring the pH to 9.0. The mixture was stored at 5° overnight when the pH had become 7.0. A trace of undissolved gel was removed by centrifuging and discarded. The supernatant solution (160 ml.) had: N, 3.43 mg./ml.; P (total), 0.25 mg./ml.; P (inorganic), 0.011 mg./ml.; S, 1.29 mg./ml.; S/N, 0.38. The original horse serum albumin solution had S/N, 0.12.

(c) *H-horse serum albumin (phosphate, pH 7.5)*. A preparation of *H*-serum albumin solution was made by reacting 63 ml. of 5.3% horse serum albumin solution, 28 ml. of 0.5M-phosphate and 3.4 ml. of *H* at 37° and pH 7.5. The pH, measured by a glass electrode inserted in the reaction vessel, was kept constant at 7.5 by the addition of NaOH. After dialysis and electro dialysis, carried out as in the above preparation, the resulting solution had $\Delta(S/N)$, 0.11 and P/N, 0.070. In this preparation 5% of the phosphate was present as inorganic phosphate.

(d) *H-guinea pig serum (no buffer, pH 8-9)*. Dialysed fresh guinea pig serum (22 ml.), water (20 ml.) and *H* (0.5 ml.) were stirred 8 hr. at 37°. The solution was kept alkaline to phenolphthalein by the addition of 2N-NaOH solution. The reaction mixture was dialysed against running tap water for 48 hr. and filtered; the product had $\Delta(S/N)$, 0.29.

(e) *Controls*. Table 1 shows the results obtained in control experiments. In each case the reactants were contained in 75 ml. water and stirred at 37° for 8 hr. at pH 8-9. The resulting solution was dialysed against running tap water for 70 hr. and then electro dialysed to zero current against distilled water.

Table 1. *Fixation of sulphur and phosphate by horse serum globulin*

Exp.	Reactants	Analyses of product	
		S/N	P/N
LXIII	Na ₂ HPO ₄ (0.0125 g.mol), horse serum globulin (2.8 g.)	0.12	0.005
LI	'Thiodiglycol phosphate' (0.0125 g.mol. P), horse serum globulin (2.8 g.)	0.12	0.041
LII	'Thiodiglycol phosphate' (0.0125 g.mol. P), horse serum globulin (2.8 g.) <i>H</i> (1 ml.)	0.24	0.062

The product of Exp. LII only was precipitated by the appropriate antiserum (Table 2).

Experiments with phosphatase. Two different phosphatase preparations were used; one was an aqueous extract of rat kidney, the other a solution of an active powder prepared from dog faeces (Armstrong, 1935). For use 10 mg. of this intestinal phosphatase preparation was dissolved in 20 ml. water. The rat-kidney extract was prepared by grinding fresh, decapsulated rat kidneys in a mortar with sand and five times their weight of water; the resultant brei was stored at 5° overnight and centrifuged. The supernatant liquid was diluted twenty times with water for use.

At pH 9.5 (veronal buffer of King & Delory, 1940) and 37° with 1 mg. of phosphate as 'thiodiglycol phosphate' and 5 ml. of enzyme solution in 15 ml. of digest, hydrolysis with the kidney phosphatase was 63% complete at 5 hr. and 100% complete at 22 hr.; hydrolysis with the intestinal phosphatase was 95% complete in 6 hr. and 99% complete in 22 hr.

Under the same conditions, with the *H*-horse serum albumin (phosphate) prepared at pH 8-9, as described in the previous section, the intestinal phosphatase gave 13% hydrolysis of phosphate at 22 hr. and the kidney phosphatase gave 7% hydrolysis at 22 hr. The *H*-horse serum albumin (phosphate) prepared at pH 7.5 on treatment with the intestinal phosphatase under the same conditions gave 28% hydrolysis of phosphate in 22 hr. Both these solutions, after treatment with intestinal phosphatase, were electro dialysed to zero current and analysed. The analyses showed that the S/N values had not been altered by the phosphatase treatment, but that the P/N values had been reduced by 20%.

The pH 8-9 preparation before and after phosphatase treatment was precipitated by antiserum and the precipitations were inhibited by 'thiodiglycol phosphate' (Tables 2 and 4).

Immunization experiments

Various courses of immunization were carried out by injecting antigens prepared by method (a) into young adult rabbits. In general, it was found that an intravenous course gave a fairly good antiserum; if this were followed by an intraperitoneal course the antiserum obtained was much weaker, but if the intraperitoneal course were followed by a second intravenous course the antiserum was once more strongly precipitating.

Antigens prepared by method (a) from a number of different serum proteins fell into the following order of effectiveness: pig serum globulin, pig serum, horse serum globulin, horse serum, rabbit serum, human serum globulin, human serum.

The above general conclusions were arrived at from experiments on 100 rabbits. As a result of these experiments, we settled on the following procedure which was expedient and almost invariably gave strongly precipitating antisera. Rabbits were given a course of four intravenous injections, each containing about 8 mg. of antigen nitrogen, at 4-day intervals, and were bled out 3-10 days after the last injection. The best results were obtained when coupled pig serum globulin prepared by method (a) was the antigen.

The immunological experiments carried out with antigens prepared by method (c) were too few to justify any conclusions. All attempts to produce satisfactory antisera for antigens prepared by method (d) failed; a large number of such attempted immunizations were made by various procedures including mixing the antigen with adjuvants according to the method of Freund & McDermott (1942).

Precipitin tests. These tests were carried out by mixing 0.5 ml. of antigen solution in increasing dilutions (1/5 to 1/2560) with 0.5 ml. of antiserum in constant dilution (1/5); the diluent was 0.85 % NaCl. All readings were made at room temperature. After the test was set up frequent readings were made to determine which tube first showed definite flocculation; this established the optimal antigen/antibody ratio. The test was then continued at room temperature overnight after which time a final reading of the precipitation was made.

Table 2 summarizes the results of precipitin tests of the sera of rabbits immunized with *H*-proteins prepared by method (a). These data are a representative selection from a large number of such tests.

Inhibition tests. A sample of strongly precipitating

rabbit serum was produced by injecting *H*-coupled horse serum globulin prepared by method (a), and this antiserum was titrated with decreasing concentrations of coupled guinea pig serum prepared by method (a). The optimum concentration of antigen for reaction with a 1/5 dilution of antiserum was 0.025 % protein. Inhibition tests were carried out on this optimal system as follows: 0.25 ml. of solutions with decreasing concentrations of the various inhibitors were incubated for 1 hr. at 37° with 0.25 ml. of a 1/2.5 dilution of antiserum; the resulting mixtures were then tested for precipitation with 0.5 ml. of coupled guinea pig serum protein solution containing 0.025 % N. The inhibitors tested in this manner were neutral solutions of thiodiglycol, thiodiglycol and sodium phosphate, 2-hydroxy-2'-glycinodiethyl sulphide, 1:4-thiazan-4-acetic acid, 'thiodiglycol phosphate' and *H*-glycine; the di-2-(2'-amino-2'-carboxyethylthioethyl) sulphide was dissolved at pH 9 since it would not dissolve at a lower pH. The only solution which produced any inhibition was that of 'thiodiglycol phosphate'; the results of a typical inhibition test with this material are to be seen in Table 3.

Table 2. *Precipitin reactions of rabbit antisera*

(The bracketed letters (a), (b), (d), (e) refer to the subsection in which the preparation of the complex is described. h.s.a. and h.s.g. = horse serum albumin and globulin, respectively; r.s. = rabbit serum; g.p.s. = guinea pig serum; p.s.g. = pig serum globulin.)

Rabbit no.	Immunizing antigen	Antiserum (1/5) titrated with	Optimal antigen conc. (% protein)	Time of appearance of ppt.	Amount of ppt.
1321	<i>H</i> -h.s.g. (a)	<i>H</i> -h.s.g. (a) (phosphate, pH 9)	0.2	2 min.	+++
1321	<i>H</i> -h.s.g. (a)	Normal horse serum	0.1	4 hr.	+
1321	<i>H</i> -h.g.s. (a)	<i>H</i> -r.s. (a) (phosphate, pH 9)	0.2	Immediate	+++
1321	<i>H</i> -h.s.g. (a)	Normal rabbit serum		No ppt.	
A24	<i>H</i> -h.s.g. (a)	<i>H</i> -g.p.s. (a) (phosphate, pH 9)	0.002	1 min.	+++
A24	<i>H</i> -h.s.g. (a)	<i>H</i> -g.p.s. (d) (no buffer, pH 9)	0.002-0.03	2 hr.	++
1353	<i>H</i> -r.s. (a)	<i>H</i> -h.s.g. (a) (phosphate, pH 9)	0.03	15 min.	+++
J74	<i>H</i> -p.s.g. (a)	<i>H</i> -h.s.a. (a) (phosphate, pH 9)	0.2	Immediate	+++
J74	<i>H</i> -p.s.g. (a)	<i>H</i> -h.s.a. (d) (no buffer, pH 9)	No ppt. Opalescence in all tubes		
A72	<i>H</i> -p.s.g. (a)	<i>H</i> -h.s.a. (b) (phosphate, pH 9, electrolyzed)	0.1	1 min.	+++
A72	<i>H</i> -p.s.g. (a)	<i>H</i> -h.s.a. (b) (same as above but after phosphatase treatment)	0.03	1 min.	+++
A72	<i>H</i> -p.s.g. (a)	<i>H</i> -h.s.g. LII (e) ('thiodiglycol phosphate'; pH 9)	0.5	1 min.	+++

Table 3. *Inhibition test using 'thiodiglycol phosphate'*

(Antiserum to *H*-horse serum globulin (phosphate, pH 9). Test antigen *H*-guinea pig serum (phosphate, pH 9). Each tube contained 0.25 ml. of antiserum (1/2.5), 0.25 ml. of inhibitor solution and 0.5 ml. of antigen (0.025 %). Degrees of precipitation: op., opalescence without particulation; tr., trace of precipitate; ±, +, ++, etc., increasing degrees of precipitation.)

Tube no.	...	1	2	3	4	5	6
Conc. of inhibitor (mg. P/ml.)		2.0	1.0	0.5	0.25	0.125	
Saline (ml.)							0.25
Precipitation observed at:							
30 min.					op.	op.	+++
2.5 hr.				op.	op.	op.	++++
6 hr.				op.	tr.	±	++++
20 hr.			tr.	+	++	++	++++

Table 4. *Effect of 'thiodiglycol phosphate' on precipitin reactions*

(Bracketed letters refer to methods of preparation.)

Rabbit antiserum to	Test antigen	Inhibition
<i>H</i> -horse serum globulin (a) (phosphate, pH 9)	<i>H</i> -guinea pig serum (a) (phosphate, pH 9)	+
<i>H</i> -horse serum globulin (a) (phosphate, pH 9)	<i>H</i> -guinea pig serum (d) (no buffer, pH 9)	-
<i>H</i> -horse serum globulin (a) (phosphate, pH 9)	<i>H</i> -rabbit serum (a) (phosphate, pH 9)	+
<i>H</i> -horse serum globulin (a) (phosphate, pH 9)	<i>H</i> -horse serum albumin (b) (phosphate, pH 9, electro dialysed)	+
<i>H</i> -pig serum globulin (a)	<i>H</i> -horse serum globulin LII (e) ('thiodiglycol phosphate', pH 9)	+
<i>H</i> -pig serum globulin (a)	<i>H</i> -horse serum albumin (a) treated with phosphatase	+
Normal horse serum	Horse serum globulin	-
Hen egg albumin	Hen egg albumin	-

The effects of 'thiodiglycol phosphate' in a number of different precipitating systems are recorded in Table 4.

DISCUSSION

The qualitative effect of the presence of sodium phosphate on the reaction of *H* with protein was first suggested by the results of the inhibition tests, although a quantitative effect had been noted previously. It had been found in our experiments that four times as much *H* could be brought into reaction with a protein in the presence of sodium phosphate to yield a soluble product as when the reaction was carried out under similar conditions but with no buffer present; this was exclusive of the amount of *H* reacting with the phosphate itself. Subsequent chemical analyses then established the fact that when *H* reacted with proteins in the presence of sodium phosphate at pH 7.5 or 9, complexes were formed which contained residues of diethyl sulphide, phosphate and protein.

The presence of ester phosphate in the *H*-protein-phosphate complexes might possibly have been due to the adsorption by the protein of some 'thiodiglycol phosphate', although this seems unlikely in view of the serological findings and the vigorous dialysis treatment which some of the complexes received before analysis. In order to obtain more information on this point, however, one complex prepared at pH 8-9 and one at pH 7.5 were treated with phosphatase. Although the two phosphatase preparations both hydrolysed the 'thiodiglycol phosphate' rapidly and completely they had only a small effect on the antigens under similar conditions.

The *H*-phosphate protein complexes are highly antigenic, and the groups introduced are powerfully determinant in the immunological sense, since such complexes formed from rabbit serum are antigenic for rabbits. There is no direct evidence for the configuration of the determinant group introduced, but one possibility is that it is the same as the 'thiodiglycol phosphate' but with protein substituted for one of the phosphate groups. In this connexion it is

important to note that some phosphate, but no sulphur, is fixed by the protein in the presence of 'thiodiglycol phosphate' and that the product does not react specifically with antiserum to *H*-protein (phosphate). This small amount of phosphate fixed in Exp. LI (Table 1) may be related to the fraction of fixed phosphate liberated by phosphatase from the complexes; in the latter case the phosphate content of the complex was reduced without a reduction in the sulphur content.

It is most unlikely that the *H*-phosphate protein complexes referred to above are the only products of reaction of *H* with protein in our experiments. A number of diethyl sulphide groups could have been introduced into the protein without the intervention of phosphate, and these could be similar to those introduced when *H* reacts with protein with no buffer present. Such a conclusion is indicated by the results obtained with antiserum A24 (Table 2). Under the conditions of our experiments, such groups showed very little capacity for producing a specific antibody response, and this lack of antigenic activity is similar to that found by Boursnell *et al.* (1946b) for their *H*-serum protein complexes. These findings make it very difficult to interpret the part played by *H*-protein derivatives in the phenomenon of hypersensitivity to mustard gas.

A number of experiments were carried out to explore the possibility that the reaction of mustard gas simultaneously with inorganic phosphate and protein was of some significance in the mechanism of action of mustard gas on living cells, but all the findings were negative. The results of our work probably have a bearing on some other biochemical investigations of the mechanism of mustard gas action such as the effects of mustard gas on enzymes and other proteins *in vitro*. In the case of phosphate, at least, the buffer could play a prominent part.

SUMMARY

1. Two pure substances, 1:4-thiazan-4-acetic acid and (probably) 2-hydroxy-2'-glycinodiethyl sulphide, have been isolated from the product of re-

action of mustard gas (*H*) with ethyl aminoacetate in anhydrous ethanol in the presence of sodium acetate.

2. The reaction of *H* with glycine in aqueous solution at pH 9 yields a water-soluble product with unusual properties.

3. *H* reacts extensively with sodium phosphate in aqueous solution; analyses of the reaction product indicate that it is a sulphonium salt derived from 2 mol. of *H* and containing three phosphate ester groups.

4. It has been found that when *H* reacts with serum proteins in the presence of sodium phosphate at pH 8–9, complexes are formed which contain, in addition to an increased concentration of sulphur, appreciable amounts of esterified phosphate which cannot be removed by electrodialysis.

5. When these complexes are injected intravenously into rabbits they give rise to strongly precipitating antisera.

6. The precipitin reactions between these coupled proteins and the appropriate antisera are inhibited by the reaction product of *H* and sodium phosphate, but not by a number of other derivatives of diethyl sulphide.

7. The complexes formed when *H* reacts with serum proteins at pH 8–9 with no buffer present have been found to be ineffective in producing serum precipitins in rabbits.

Microanalyses of the reaction products of mustard gas and ethyl aminoacetate were carried out by Mrs Barker through the kindness of Dr Leo Marion, Chemistry Division, National Research Council, Ottawa. Carbon and hydrogen contents of the 'thiodiglycol phosphate' were measured by Dr A. Elek, Los Angeles, California.

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Investigations into Cholinesterase Levels in Serum and Cerebrospinal Fluid of Psychotic Patients

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Claims have been made that the level of serum cholinesterase can be correlated with certain psychiatric conditions. This level is said to be raised in states of anxiety and depression, and lowered in catatonic stupor, schizophrenia and epileptic conditions (Jones & Tod, 1937; Jones & Stadie, 1939; Birkhäuser, 1941*a, b*; Richter & Lee, 1942; Butt, Comfort, Dry & Osterberg, 1942; Schütz, 1944).

The data accumulated were quite often inconsistent and did not permit definite conclusions about the role of cholinesterase in disturbances of the nervous tissue. Mendel, Hawkins & Nishikawara (1948) assumed that this might be due to the fact that at the time when these earlier investigations were reported the existence of two enzyme systems

capable of hydrolysing acetylcholine was not known. Only later Mendel & Rudney (1943) and Mendel, Mundell & Rudney (1943) distinguished between the true cholinesterase and the pseudo-cholinesterase and developed methods for measuring the activities of these enzyme systems. The authors claimed that only the true cholinesterase is essential for hydrolysis of acetylcholine *in vivo*.

The investigations reported in this paper were made because of certain therapeutic possibilities envisaged, which might occur when using different substances influencing cholinesterase activity. The following points were of particular interest: (i) Whether there existed any correlation between the values of true cholinesterase and the total brain size