is greatly reduced, though a small amount should occur in the case of nearly perfect collisions.

The effect of interposing a foreign substance, such as C_2H_5OH in the case of CBr_4 none of whose atoms have masses near that of the active atom, obviously would be to slow the radioactive atom so that when it finally did make a collision with an atom of nearly its own mass it would have insufficient energy to rupture the bond in the "billiard ball" fashion described above. This of course results in nearly zero retention.

The principles outlined seem to be generally applicable. In review:

1. Retention of activity occurs mainly in the target substance.

2. Dilution with a solvent none of whose atoms are near the active atom in mass results in the limit in zero retention.

3. Retention in the gaseous state is much lower than in the liquid or solid state. The limiting retention at zero pressure is not necessarily zero, but may be a few per cent., depending on the molecule. Of course the reformed molecule is usually somewhat excited and may be able to take part in reactions which the normal molecule could not. These reactions may be used to liberate the active atom also. For example, Lu and Sugden[2] have shown that the addition of aniline to brombenzene reduces the retention very considerably, probably by the reaction

$$C_6H_5Br^* + C_6H_5NH_2 = Br^{\pm} + (C_6H_5)_2\overset{+}{N}H_2$$

resulting in the liberation of the active bromine atom.

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THE EFFECT OF HEAT ON CRYSTALS OF SERUM ALBUMIN; PRODUCTION OF CRYSTALS OF DENATURED PROTEIN

A STUDY of the effects of heat on crystals of serum albumin has led to some curious and unexpected results. That heat denatures proteins is well known, and many investigations have been made on the heat denaturation of protein *solutions*. For a study of heat denaturation of proteins *in the crystalline state* horse serum albumin is a suitable protein because crystals of this protein do not tend to dissolve when the temperature rises, as crystals of egg albumin, for example, do. On the contrary, under certain conditions, warming a solution of horse serum albumin increases the rate of crystallization. If 0.5 ml of serum albumin solution (containing 50 mg of protein, previously crystallized, but not fractionated and subsequently dialyzed free of salt and then filtered) is added to 12 ml of a sodium sulfate-acetate solution (made by mixing 10 ml of 23 per cent. sodium sulfate with 1 ml of 2 M sodium acetate and 1 ml of 2 M acetic acid), crystallization begins in less than 5 minutes if the solution is warmed to 45° C., and within 15 minutes there appears a plentiful crop of large well-formed, needle-shaped crystals. At 25° crystallization proceeds much more slowly. These crystals dissolve at once if 12 ml of water are added. Heating the original solution of serum albumin in sodium sulfate-acetate to 60°, instead of 45°, causes a large flocculent precipitate of protein to appear almost at once. This precipitate is amorphous and, if heating at 60° is continued for 15 minutes, practically no protein dissolves when the suspension is subsequently cooled to room temperature and 12 ml of water are added. At 60° the amorphous protein precipitate obtained is obviously denatured.

At this point it seemed of interest to inquire what would happen to crystals of serum albumin if they were heated to 60° and still higher temperatures. For this purpose crystallization at 45° was allowed to proceed for several hours. The crystals were separated by centrifuging from the small amount of albumin still remaining in solution, and to the crystals was added the original volume of sodium sulfate-acetate mixture at 45°. In this medium the protein crystals were heated at various temperatures from 60° to 100° and finally in an autoclave at 115°, at each temperature for 15 minutes. At no temperature were the crystals destroyed. Even after heating at 115° the crystals seemed as perfectly formed as before heating.

The solubility of the heated albumin crystals was tested at room temperatures by adding to each heated preparation 2 volumes of water. The crystals heated at 60° dissolved in the course of five or ten minutes. Since heating a *solution* of serum albumin under the same conditions renders the albumin insoluble, it is clear that the protein in the crystal is not as easily denatured as is dissolved protein. If protein denaturation is an unfolding process, as there is good reason to believe,[1] then the increased stability of the protein in a crystal may be explained by supposing that the tendency of a molecule to unfold as the temperature is raised is opposed by the bolstering effect of neighboring molecules in the crystal.

Crystals of serum albumin heated at temperatures higher than 60° did not dissolve completely. A small percentage of those heated at 70° dissolved, but practically none of the crystals heated between 80° and 115° dissolved even after standing, with occasional stirring, for three days. These albumin crystals were not destroyed by being placed for several days in 1 N HCl or 95 per cent. alcohol. They dissolved at once, however, in a saturated urea solution. The insoluble serum albumin crystals are as insoluble as a heat-denatured, amorphous coagulum of serum albumin.

[1] A. E. Mirsky and L. Pauling, *Proc. Nat. Acad. Sci.*, 22: 439, 1936.

[2] C. S. Lu and S. Sugden, *Jour. Chem. Soc.*, 1273, 1939.

[3] W. F. Libby, *Jour. Am. Chem. Soc.*, 62: 1930, 1940. This paper contains numerous other references of importance.

One hesitates, however, to compare the heated crystals with a heat denatured protein because, despite many efforts, no denatured protein has been crystallized. And yet it can be shown that the heated, insoluble crystals are indeed crystals of denatured protein. The crystals, washed free of sodium sulfate by repeated centrifugation, readily dissolve in a pH 9.2 borate buffer. Crystals prepared from 0.3 ml of the serum albumin preparation mentioned above can be dissolved in 0.4 ml of a 0.1 M pH 9.2 borate buffer. If to this solution at 45° are added 5 ml of the sodium sulfate-acetate mixture used for crystallizing serum albumin, no crystals form. Instead all the protein immediately precipitates amorphously, and this precipitate does not dissolve when the salt solution is diluted with an equal volume of water. The albumin dissolved by placing crystals previously heated at 80° in the pH 9.2 borate buffer has the characteristic properties of a denatured protein. Denaturation is not caused by the pH 9.2 buffer, for if this buffer is added to native, unheated serum albumin, there is no difficulty in crystallizing the albumin and subsequently dissolving the crystals in water.

It is clear, then, that the heated crystals of serum albumin that are insoluble in water are crystals of denatured protein. Denaturation does not destroy the crystal pattern (although crystallographic analysis will probably show that it has been changed) but once the denatured albumin molecules are released from their confinement within the crystal by being dissolved in a pH 9.2 buffer it is impossible to replace them in the ordered pattern characteristic of a crystal of native protein. It is possible to obtain crystals of denatured protein by denaturing a protein while it is in the crystalline state, but it does not seem to be possible to crystallize a denatured protein.

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N^1-(β-AMINOETHYL)SULFANILAMIDE AND N^1-(β-DIETHYLAMINOETHYL) SULFANILAMIDE

THESE compounds were prepared from monoacetylethylenediamine[1] and β-diethylaminoethylamine,[2] respectively. The amine in an aqueous solution containing 1.5 molecular proportions of sodium bicarbonate was shaken for five hours (ten hours for the second compound) with a chloroform solution of 1.2 molecular proportions of acetylsulfanilyl chloride.[3] The insoluble material was separated by filtration (in the case of the second compound, after evaporating off most of the water and chloroform) and was hydrolyzed by boiling with 6 normal hydrochloric acid (8 cc per gram of the precipitate; in the case of the second compound, 4 cc per gram of the precipitate) under a reflux condenser for twelve hours. The compounds were isolated as the dihydrochlorides by evaporation of the solutions to dryness with a current of warm air and were purified by crystallization from ethyl alcohol-water mixtures (85 per cent. alcohol for the first compound; 95 per cent. for the second).

N^1-(β-Aminoethyl)sulfanilamide dihydrochloride. Yield (based on monoacetylethylenediamine): 90 per cent. Calculated for $C_8H_{15}O_2N_3SCl_2$: N, 14.58 per cent.; Cl, 24.61 per cent. Found: N, 14.37; Cl, 24.04. M.p. 217–220°[4].

N^1-(β-Diethylaminoethyl) sulfanilamide dihydrochloride. Yield (based on β-diethylaminoethylamine): 30 to 65 per cent. Calculated for $C_{12}H_{23}O_2N_3SCl_2$: N, 12.20 per cent.; Cl, 20.60 per cent. Found: N, 12.03; Cl, 20.59. M.p. 190–195°[4].

The synthesis of additional N^1-(β-dialkylaminoethyl) sulfanilamides is in progress. These compounds will be tested for chemotherapeutic activity.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

ANOTHER CIRCUIT FOR TEMPERATURE. CONTROLS

NUMEROUS articles have appeared in the literature recently describing circuits intended for use with thermostatic devices to control closely the temperature of ovens and chambers used for biological and chemical processes. Two of these have appeared within the last three months in SCIENCE.[1, 2]

Most of the circuits so far described require the use of one or more thermionic tubes to amplify the current passing through the control device. This greatly amplified current is caused to operate a commercial relay. The satisfactory use of these tubes often involves the use of transformers, condensers and numerous resistances. Occasionally it has been found that changes in atmospheric conditions alter the values of

[1] A. C. Hall and L. J. Heidt, SCIENCE, 92: 2380, 133, August 9, 1940 and SCIENCE, 92: 2400, 612, December 27, 1940.

[2] Charles Butt, SCIENCE, 92: 2389, 339, October 11, 1940.

[1] Prepared by the method of Arthur J. Hill and Samuel R. Aspinall, *Jour. Am. Chem. Soc.*, 61: 822–5, 1939.

[2] Prepared as described by Lawrence H. Amundsen and Karl W. Krantz, *Jour. Am. Chem. Soc.*, 63: 305–7, 1941.

[3] Prepared by the method of S. Smiles and Jessie Stewart, "Organic Syntheses," collective vol. 1, edited by Henry Gilman, pp. 8–9. New York: John Wiley and Sons, 1932.

[4] Melting point ranges were somewhat indefinite. The compounds seemed to be undergoing decomposition, as gas appeared to be evolved.