ON THE RELATION OF NECROSIS AND INFLAMMATION TO DENATURATION OF PROTEINS

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Plates 52 to 54

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The present study has been suggested by earlier experiments (1) in which the molar concentration of electrolytes isotonic with slices of liver or of kidney has been found to be determined by the molecular weight, valence, and ion dissociation of these electrolytes. This relation is in accord with freezing point depression, vapor tension, electric conductivity, and other colligative properties of electrolytes. Contact of cells with solutions of electrolytes soon may cause such injury to them that the semipermeability of their plasma membranes is impaired. The possibility that tissue injury by electrolytes varies with their molecular conformation has suggested the possibility that tissue injury during life might be referable to denaturation of the protein constituents of cells or of intercellular substance.

Denaturation of proteins caused by the action of heat, solutions of acids or alkalis, electrolytes, alcohols, and some other agents produces well known changes in the solubility of albumins and globulins, especially evident at their isoelectric point. Denatured proteins may show increased susceptibility to the action of digestive enzymes—a property which gives increased digestibility to protein foods that have been cooked. Sulfur groups which are firmly bound and hidden within the protein molecule may be liberated so that they give identifying reactions.

Hardy (2) found that colloid particles of protein in a boiled solution of egg white under the influence of a constant electric current moved with the negative stream when the reaction of the fluid was alkaline or with the positive stream when it was acid; they aggregated to form a coagulum in the one or other direction (2). Denatured protein which is soluble at the isoelectric point of the protein is made insoluble by acid or alkalis. Denaturation of protein, as usually defined, designates the changes that precede coagulation.

Denaturation varies much with the diverse physical and chemical agents that cause it (3). Furthermore, it varies with the character and concentration of protein solutions (4, 5). In spite of these limitations it has been regarded as one of the most significant changes undergone by proteins (3) and it has been studied with the purpose of obtaining insight into their structure.

Plan of the Experiments

The characteristics of tissues that have undergone necrosis, that is, local death within the living body, indicate that their protein composition has undergone profound changes. The infarction of tissue by the occlusion of its blood vessels and the necrosis of liver parenchyma, of kidney cortex, or of intestinal mucosa caused by chemical injury is accompanied by changes long ago designated as "coagulative necrosis." Visible localized necrosis of the skin has offered opportunity to control its production. When solutions of various substances are injected into the dense tissue of the dermis they do not spread widely and their removal by blood vessels and lymphatics is retarded. A small quantity of fluid, approximately 0.1 cc injected into the dermis, produces a dome-like bleb about 8 mm in diameter. It remains for a time and gradually subsides. When severe injury is caused by the injected solution, the skin in a well defined area may assume a gray color and become somewhat rigid. Within 24 hours various substances in appropriate concentration cause necrosis in an area seldom exceeding 1 cm in diameter, but usually less (See Figs. 1 to 12). A defect in the skin is covered by a slightly raised reddish brown scab. About its margin is a well defined, pale, almost white, slightly raised zone about 1 mm wide. It is surrounded by a less definite but broader zone of congestion. The scab surrounded by a pale, sharply defined zone with an outer diffuse zone of congestion is a characteristic lesion.

Method

White rats have been used in these experiments. The hair over the abdomen has been carefully removed by means of electric clippers; shaving of the skin with a razor might cause some irritation and injury. The skin surface has been rapidly cleaned with alcohol promptly removed with cotton gauze, and injections have been made with precautions for asepsis. Animals have been lightly anesthetized with ether, when multiple injections have been required. The point of a fine needle (26 gauge on a 0.5 cc syringe) has been inserted into the dermis parallel with the skin surface for a distance of about 3 mm. Tests on each animal have usually been made with 4 or more solutions of graded molar concentration at approximately the same distance from the midline and by repeated experiments have determined the least concentration that causes necrosis. Water or physiological salt solution in equal quantity (0.1 cc) has been injected occasionally for control.

Lesions in width from 10 mm up to 5 have been recorded in the tables as ++; those 5 mm or less as +, and no apparent injury as −.

Effect of Acids and Alkalis

When 0.1 cc of distilled water is injected into the dermis the skin surface on the following day is slightly elevated in an area about 3 mm diameter and there is slight palpable induration of the skin. Later a faintly red spot may remain during several days but there is no evidence of local injury (spot *d* of Fig. 12).

Acids and alkalis have been injected into the dermis in volume of 0.1 cc with graded normal concentrations (Table I). Hydrochloric acid and sodium hydroxide in solutions 0.1 and 0.01 normal have caused necrosis of skin. It has also been caused by ammonium hydroxide in a

more dilute solution (Fig. 1). The proteins that have been found in skin have isoelectric points between 4.7 normal (albumin) and 6.8 normal (hemoglobin) (6). Hydrochloric acid with approximate hydrogen-ion concentration of pH 1 and 2 and sodium hydroxide solutions with pH 10–13 have caused necrosis but none has occurred in solutions between these ranges (Table I).

TABLE I

Necrosis of Skin Caused by Acids and Alkalis

Acids and alkalis have been placed from left to right in order of their hydrogen ion concentration, pH 1–13. + indicates that the corresponding concentration caused necrosis of the skin; − indicates that none was found.

	Per cent of normal hydrochloric acid				Water	Per cent of normal sodium hydroxide			
Injected into dermis in volume of 0.1 cc.	0.1	0.01	0.001	0.0001		0.0001	0.001	0.01	0.1
Necrosis of skin	+	+	−	−	−	−	−	+	+
Hydrogen ion concentration	1	2	4	6		8	10	12	13

	Per cent of normal ammonium hydroxide			
	0.0001	0.001	0.01	0.1
	−	+	+	++

Effect of Electrolytes

The destruction of colloid solutions by salts of metals was first investigated by H. Schulze (7) who showed that this property increased with the valence of the basic ion. Hardy and Neville (8) studied the coagulation of various colloid solutions by salts and found that this power was determined chiefly by the valence of the ion of the metal. They believed that the valence of the ion of the acid had some but less effect.

Under the conditions of the present experiments all of the electrolytes that have been tested have caused necrosis of the skin when introduced in concentrations of 2 molar or more (Table II). The minimum concentration necessary to produce this effect has decreased with increase of valence of the cations of these salts. With sodium, potassium, and ammonium, representing monovalent ions, necrosis has followed the injection of solutions 1 or 2 molar; with magnesium and with calcium, having bivalent ions, it has occurred with solutions 0.5 and 0.01; with trivalent ions of lanthanum and of aluminum necrosis has been caused by 0.005 and 0.001 molar.

These experiment show that the quantity of different electrolytes necessary to produce necrosis of the skin vary with the valence of their basic ions, on the one hand, and, on the other, with the molar concentration of the corresponding electrolytes found in preceding experiments (9) to be in osmotic equilibrium with cells of the liver and of the kidney and in the same order.

The corresponding order of minimum concentration causing skin necrosis

TABLE II

Necrosis of the Skin Caused by Electrolytes

Molar concentration	0.5	4.0	3.0	2.0	1.0	0.5	0.1	0.05	0.01	0.005	0.001
Sodium chloride	++	++	+	+							
Sodium sulphate			++	+							
Sodium nitrate			++	+	+						
Sodium citrate					+	+					
Potassium chloride		++		++	+						
Potassium sulphate						+					
Potassium nitrate				++	++	+					
Potassium citrate				++	++	+					
Ammonium chloride		++		++	+						
Magnesium chloride					+	+					
Magnesium sulphate						+					
Calcium chloride				++	++	++	++	+	+		
Lanthanum chloride					++	++	+		+	+	
Lanthanum nitrate						++	++		+	+	
Aluminum chloride									++	++	+

and of osmotic equilibrium in relation to valence is illustrated by chlorides with cations of increasing valence.

	Molar concentration		
	Causing necrosis	Isotonic with liver cells	Isotonic with kidney cells
NaCl	2.0	0.34	0.23
KCl	2.0	0.35	0.30
NH_3Cl	1.0	0.31	0.24
$MgCl_2$	0.5	0.18	0.19
$CaCl_2$	0.01	0.22	0.17
$LaCl_3$	0.005	0.14	0.09
$AlCl_3$	0.001	0.16	0.11

Variation in the denaturation of protein corresponding with the valence of the acid ion of electrolytes has been observed by Hardy and Neville (8), but it has been found much less than that determined by valence of basic ions. A similar relation to necrosis is indicated by two experiments in each of which electrolytes with the trivalent acid ion of a citrate has caused more necrosis than the monovalent ion of a nitrate, the associated basic ion being the same in each instance. Exact comparison is possible because corresponding injections have been made in the same animal. Necrosis caused by sodium citrate (Fig. 3) with trivalent acid ion has been greater than that with sodium nitrate with

monovalent acid ion and the same relation is seen with the potassium salts of these two acids (Fig. 4).

Effect of Heavy Metals

Necrosis of the skin has been caused by salts of heavy metals in dilutions greater than those of any of the electrolytes listed in Table II, save aluminum which as an element has characteristics similar to those of the heavy metals. Mercuric chloride and silver nitrate have caused necrosis of the skin in dilutions of 0.0015 molar (Table III). Copper chloride has had similar effect in dilutions of 0.015 molar.

TABLE III

Necrosis of Skin Caused by Heavy Metals

Molar concentration	0.15	0.015	0.0015	0.00015	0.00015
Mercuric chloride		++	++	−	−
Silver nitrate		+	+	−	−
Copper chloride	++	+	−	−	

TABLE IV

Necrosis of Skin Caused by Amino Acids and Related Compounds

Molar concentration	8	6	4	2	1	0.5	0.15	0.1	0.05	0.01	0.001	0.001
Ammonium hydroxide								++		+	+	−
Histamine					++	++	++	+	+	+	−	−
Arginine							+	+	+	+		
Choline chloride			++	++	++	−						
Methyl urea			++	−	+	−						
Urea	++	+	+	−								

The amino acids, histamine, and arginine have caused skin necrosis in dilute solution, namely 0.01 molar—somewhat less than that of the dilution of ammonium hydroxide which has the same power. Table IV records necrosis following injection of substances characterized by the amino group and shows necrotizing power diminishing from that of histamine (Figs. 8, 9, and 11) and arginine (Fig. 7) to urea (Figs. 5 and 6). The latter has not caused necrosis in dilutions less than 4 molar.

The quantity of amino compounds needed to produce skin necrosis has a diminishing sequence which follows the same order as molar concentrations of

the same substances found to be isotonic with liver tissue in foregoing experiments (9).

	Molar concentration causing necrosis of skin	Molar concentration isotonic with liver
Histamine	0.01	0.29
Arginine	0.01	0.26
Choline chloride	1.0	1.28
Methyl urea	1.0	1.26
Urea	4.0	1.56

The relation of necrosis to isotonicity is significant because liver cells act as osmometers (1). The figures repeat the similar relation found between skin necrosis caused by electrolytes and molar concentration of solutions of corresponding substances isotonic with liver and with kidney.

TABLE V

Necrosis of Skin Caused by Oils

cc.	0.05	0.01	0.0075	0.005	0.0025	0.001	0.001
Mustard oil	++	+					
Oil of turpentine		+	+				
Croton oil		++				++	++

Effect of Oils

Overton (10) long ago assembled evidence showing that the lipoid contents of the cell surface permitted the penetration of substances soluble in lipids. Bland oils, as mineral oil or olive oil, produce scant reaction when injected into the interstitial tissue (Hass, 11) and provide a medium by which small quantities of more potent substances may be diluted. The minimal quantity that causes skin necrosis has been determined by injection of the designated quantity into the dermis with volume brought to 0.1 cc by olive oil.

The smallest quantity of oil of turpentine which has produced necrosis when injected in this same volume has been 0.0075 gm (Table V). A conspicuous feature of this necrosis is the inflammatory reaction which occurs about it, characterized by edematous swelling and induration. These early changes are soon followed by the appearance of yellowish white spots of suppuration surrounding and overlying the necrotic tissue.

Mustard oil has less necrotizing power than oil of turpentine and croton oil much more (Fig. 1 *d*.) Castor oil (Fig. 10) has caused inflammation with no evident necrosis.

Effect of Halogen Substitution Compounds of Methane

Carbon tetrachloride, chloroform, and methylene chloride are soluble in lipoids and almost insoluble in water. Necrosis of the skin produced by them has special interest because Graham (12) showed that these and other halogen substitution products of methane, when given to rabbits, cause necrosis about the central veins of the liver lobules. When chloroform, carbon tetrachloride, and methylene chloride were dissolved in olive oil they caused skin necrosis in quantities of 0.01, 0.02, and 0.005 cc, respectively (Table VI, Fig. 12).

TABLE VI

The Quantity of Halogen Substitution Products of Methane Causing Skin Necrosis when Dissolved in 0.1 cc of Olive Oil

cc.	0.05	0.03	0.02	0.01	0.005	0.001
Chloroform	++			+	−	−
Carbon tetrachloride	++	++	++	−		
Methylene chloride	++	++	++	++	+	−

The Relation of Inflammation to Necrosis

Changes caused by injury of the skin are in considerable part dependent upon the vascular supply of the tissue. Arterial branches pass through the subcutaneous tissue and form an abundant network just below the platysma which, in the rat, forms a thin but well defined layer of striated muscle fibers within the subcutaneous tissue. On the outer side vertical or tangential branches form a second network immediately below the papillae. Necrosis and inflammation produced by different agents vary in extent but have similar character. The necrotic tissue has the homogeneous acidophile appearance often designated as coagulative necrosis; nuclei have disappeared. This change may be limited to the epithelium and the immediately adjacent part of the papillary layer but may extend throughout the dermis to include the platysma and adjacent part of the subcutaneous tissue. Inflammatory changes, that is, edema and accumulation of leucocytes, are usually most evident in the neighborhood of the two vascular networks. Inflammation occurs in the vascularized tissue adjacent to sites of necrosis.

Inflammation in the present experiments is especially conspicuous when caused by substances that have been much used in the experimental study of inflammation, such as silver nitrate, soluble in water, and turpentine oil, soluble in lipids. Both in high dilution cause necrosis and inflammation. In still higher dilution they produce evident inflammation with conspicuous accumulation of leucocytes in the absence of evident necrosis. These changes

may be explained by the assumption that denaturation of protein brings about increased permeability of blood vessels when the concentration of the injurious agent is insufficient to destroy the tissue.

The Changes in Skin Immersed in Solutions that Denature Proteins

When skin, including both dermis and epithelium, is carefully separated from the subcutaneous fat and immersed in solutions of substances used for the fixation of tissues, the familiar changes occur. After 3 hours of immersion in solutions of mercuric chloride or of silver nitrate, with concentrations of 0.015 or 0.0015 molar, skin becomes rigid and has the usual appearance of fixed tissue. These same solutions cause skin necrosis *in vivo* (Table III). When immersed in 0.01 normal hydrochloric acid, skin takes on the appearance of fixed tissue and in a 0.01 molar solution it is soft and gelatinous; both solutions cause skin

TABLE VII

Solubility of Extracted Proteins of Skin Denatured by an Acid and an Alkali

Extract from skin in 0.15 molar sodium chloride	Percent of normal solutions of hydrochloric acid				Sodium chloride 0.15 molar	Per cent of normal solutions of sodium hydroxide			
	0.05	0.01	0.001	0.0001		0.0001	0.001	0.01	0.05
Turbidity with acid and with alkali	100	122	70	46	70	100	172	182	192
Hydrogen ion concentration	1	2	4	6		8	10	12	13

necrosis. In 0.15 molar solutions of histamine pieces of skin become swollen and surrounded by gelatinous material, and the solution causes skin necrosis *in vivo*.

More interpretable information is obtained when the solubility of proteins obtained from the skin is measured (Table VII).

Skin from the ventral surface of the body of a white rat has been immersed in sodium chloride solution, 0.15 molar, and scraped with a knife so that an opaque suspension containing particles and extracted material is obtained. About 3 gm of skin has been extracted with 100 cc of the salt solution and divided into portions of 10 cc. These have been added in the same quantity to graded solutions of acid and alkali adjusted to make the concentrations given in Table VII. Sodium chloride has been added to maintain a concentration of 0.15 molar. The turbid suspensions have been kept during 3 hours at 37°C and then filtered through ash-free filter paper. Relative protein content of these solutions has been determined by the turbidity method of Kunitz and Northrop (13). Equal parts of 5 per cent trichloroacetic acid made up in 0.25 saturated solution of ammonium sulphate have been added and turbidity has been measured in a photoelectric colorimeter.

The greatest solubility of protein (Table VII) has been in the more concentrated acid or alkali and less between pH 4 and 8. It is noteworthy that the

proteins of the skin, which, save for collagen, are in small quantity, have isoelectric points varying from pH 4.7–5.4 (6) and in this range, as Table VII shows, solubility has been least.

RECAPITULATION AND DISCUSSION

Necrosis of the skin caused by the injection of a measured quantity of various solutions offered opportunity to observe the occurrence and severity of necrosis in a living tissue. Since these injections were made into the interstitial tissue of the dermis it can be assumed that the temperature, hydrogen-ion concentration, and electrolyte content of the surrounding living tissue remained approximately constant save for changes induced by the solutions at the site of introduction. The injected volume of 0.1 cc was held for a time within the dense tissue of the dermis and necrosis, when it occurred, was recognizable within 24 hours or less. The injected material had been in contact with the proteins of the skin and the experiments made with a considerable variety of substances, including acid and alkalis, salts of electrolytes, and amino acids, together with some related substances have indicated that necrosis of the living tissue has occurred with definable relation to denaturation of the proteins of the tissue.

McLaughlin and Theis (14) have described the proteins of the skin as follows: albumin and globulin, very soluble in physiological salt solution, forming 32.16 per cent of the weight of the skin of the cow; mucoid, soluble in half-saturated calcium hydroxide and precipitable by acetic acid at the neutral point, forming 13 per cent; elastin, soluble in hot water and in dilute acid and alkalis, 0.1 per cent; collagen, very slightly soluble in cold water or in dilute solutions of acids or alkalis, 30.8 per cent.

When graded concentrations of hydrochloric acid and of sodium hydroxide were injected into the skin (Table I) necrosis occurred with solutions 0.1 and 0.01 normal of both acid and alkali. With concentrations 0.1 and 0.01, acidity was pH 1 and 2 and with alkalinity pH 12 and 13 (Table VII). In these concentrations of acid and alkali proteins undergo precipitation and denaturation. It can be assumed that none occurred at the isoelectric points of the proteins that were present in the tissue. The isoelectric point of serum albumin is pH 4.7 and of serum globulin 5.4.

What part collagen—which is almost insoluble in water or in physiological salt solution—takes in protein denaturation of the tissue is questionable. It is changed to form gelatin in the presence of both acid and alkalis and this change was evident in skin immersed for 3 hours in the more concentrated solutions of hydrochloric acid and of sodium hydroxide, but absent in solutions approximating the isoelectric point of collagen, namely pH 4.8 (Porter, 15). Elastin, like collagen, is almost insoluble in water. Albumins and globulins, in so far as present in fibroblasts and endothelial cells, doubtless undergo this

change. Mucoid protein from the vitreous humor has been found to undergo denaturation (Reiss and Roche, 16). The proteins of the blood, lymph, and interstitial fluid, including serum albumin, serum globulin, fibrinogen, and hemoglobin are susceptible to the same change.

An important characteristic of the coagulation and denaturation of proteins by electrolytes is its relation to the valence of the basic ion of these salts. The concentration of electrolytes needed for denaturation increases with the valence of the cation of the electrolyte. The experiments that have been described show that the potency of the electrolyte, measured by the minimal quantity necessary for the production of necrosis, similarly increases with the valence of the cation of the electrolyte.

The molar concentration of electrolytes that is isotonic with cells of liver and of kidney was found in preceding experiments (1) to be dependent upon the osmotic activity of the cell, as measured by the molecular weight, valence, and ion dissociation of these electrolytes. When the power of various electrolytes to produce necrosis is compared, it too is found to increase in the order of the valence of their basic element. The level of molar concentration that is isotonic with cells of liver or of kidney decreases in the same order. It seems probable that the two changes are dependent upon similar factors. This opinion is supported by the evidence that necrosis caused by amino compounds had a similar relation to osmotic changes in the cells. In accord with this evidence the essential feature of electrolytes and of amino compounds that determines both is apparently necrotizing power and osmotic activity is molecular structure and ion dissociation.

Inflammation has been found associated in all instances with the necrosis caused by acids and alkalis, electrolytes, and amino compounds. Substances of these sorts used in the present experiments have been soluble in water. The experiments include some made to determine if substances that are insoluble in water produce necrosis. Among these substances are agents which in the past have been much used for the experimental study of inflammation. Turpentine and croton oil dissolved in a bland oil such as olive oil caused necrosis when injected into the dermis and this necrosis was associated with inflammatory changes, as indicated by edema and accumulation of leucocytes. When injected in quantity slightly less than that which produced demonstrable necrosis, inflammation alone was evident. The presence of lipoid substances in endothelial cells, fibroblasts, and other cells explains the diffusion of these lipoid agents and the production of the associated inflammation. A group of substitution products of methane have been similarly tested because they are are lipoid-soluble and have been widely used in experimental studies of liver necrosis. Carbon tetrachloride, chloroform, and ethylene chloride in very small quantities dissolved in olive oil produced skin necrosis and inflammation.

SUMMARY AND CONCLUSIONS

Necrosis of the skin was produced by the injection of measured quantities of electrolytes and of amino compounds into the dermis, and the relative ability of these substances to produce it was determined. Inflammation characterized by edema and accumulation of leucocytes accompanied necrosis.

The ability of electrolytes to produce necrosis was found to increase with the valence of their basic ion, and in this respect was in accord with their ability to denature proteins.

The quantity of different electrolytes needed to produce necrosis varied in the same order as the molar concentration of these electrolytes, that is isotonic with liver or kidney cells. Necrosis caused by amino compounds occurred with similar relation to the isotonicity of liver cells. In this as in other relations the cells acted as osmometers.

The foregoing relations indicate that denaturation of proteins, necrosis of living tissue, and osmotic activity of liver or kidney cells are determined by molecular weight, valence, and ion-dissociation of electrolytes, that is, by the factors that determine the colligative properties of electrolytes.

Agents such as turpentine, mustard, or croton oil and some halogen substitution compounds of methyl that are insoluble in water and soluble in lipoids have produced skin necrosis and inflammation.

These experiments have been made with the able assistance of Miss Maria Tershakovec. The photographic records have been made by Mr. William Sliva.

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EXPLANATION OF PLATES

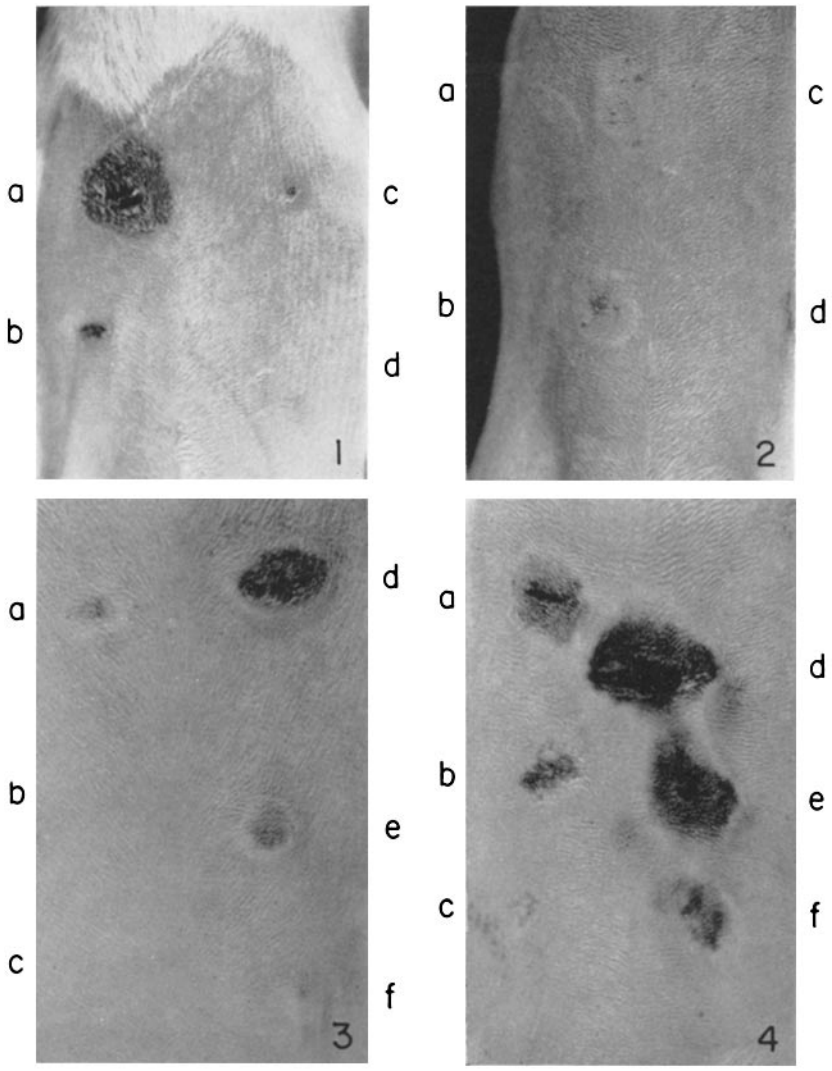
PLATE 52

FIG. 1. Necrosis of the skin 2 days after the injection of 0.1 cc of solutions of ammonium hydroxide into the dermis: (*a*) approximately 2.9 per cent, ++; (*b*) 0.29 per cent, +; (*c*) 0.029 per cent, +; and (*d*) 0.0029 per cent, −.

FIG. 2. Necrosis 1 day after the injection of 0.1 cc of solutions of sodium chloride: (*a*) 5 molar, ++; (*b*) 3 molar, +; (*c*) 1 molar, −; and (*d*) 0.5 molar, −.

FIG. 3. Necrosis 1 day after injection of 0.1 cc of sodium nitrate: (*a*) 2 molar, +; (*b*) 1 molar, −; (*c*) 0.5 molar, −; sodium citrate: (*d*) 2 molar, ++; (*e*) 2 molar, ++; and (*f*) 0.5 molar, +.

FIG. 4. Necrosis 1 day after injection of 0.1 cc of potassium nitrate: (*a*) 2 molar, ++; (*b*) 1 molar, ++; (*c*) 1 molar, +; potassium citrate: (*d*) 2 molar, ++; (*e*) 1 molar, ++; and (*f*) 0.5 molar, ++.



(Opie: Denaturation of proteins)

PLATE 53

FIG. 5. Necrosis 1 day after injection of 0.1 cc of urea: (*a*) 8 molar, ++; (*b*) 4 molar, +; and (*c*) 2 molar, −.

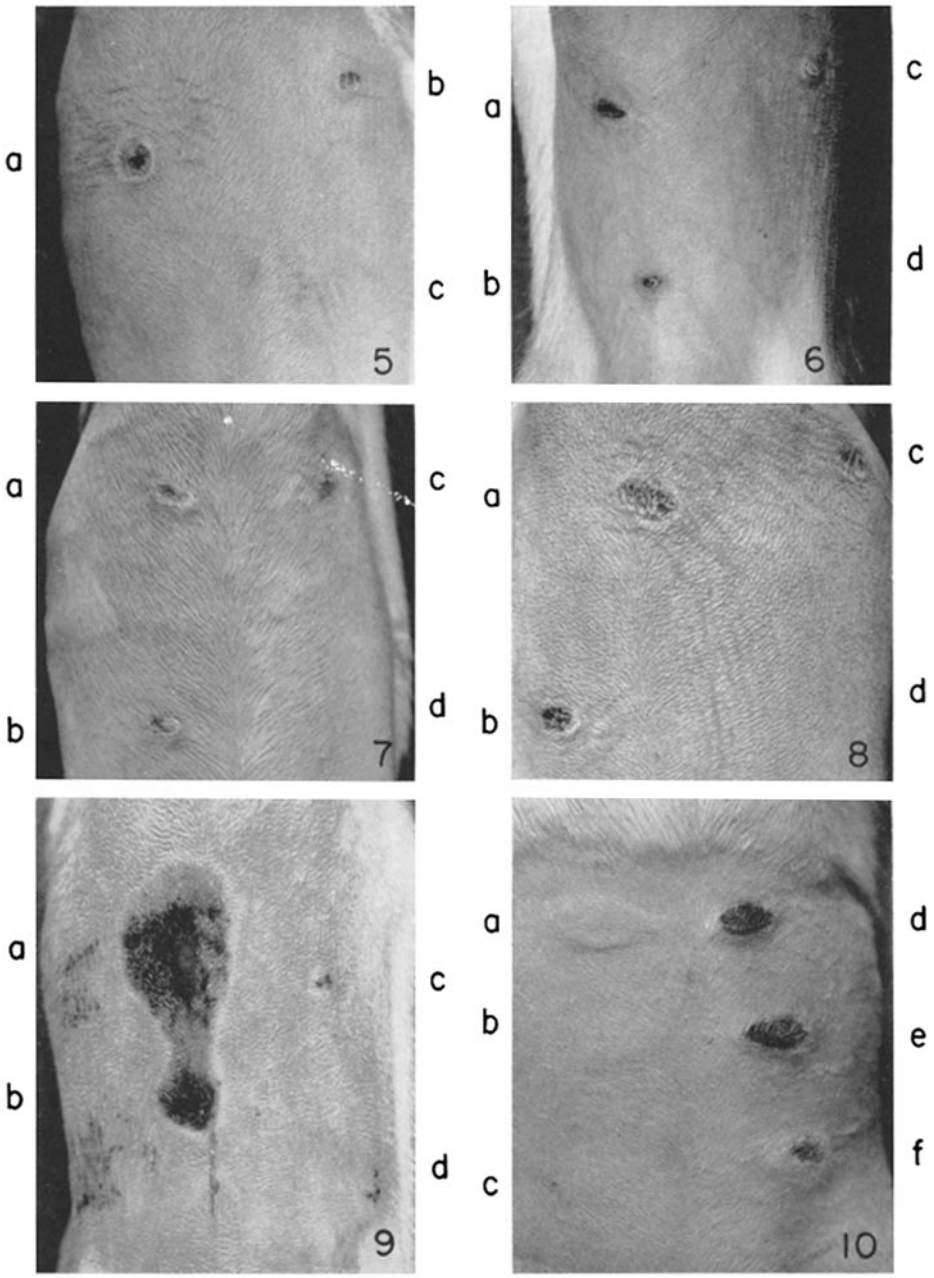
FIG. 6. Necrosis 8 days after injection of 0.1 cc of urea: (*a*) 8 molar, ++; (*b*) 6 molar, +; (*c*) 4 molar, +; and (*d*) 2 molar, −.

FIG. 7. Necrosis 1 day after injection of 0.1 cc of arginine: (*a*) 0.15 molar, +; (*b*) 0.1 molar, +; (*c*) 0.05 molar, +; and (*d*) 0.01 molar, −.

FIG. 8. Necrosis 1 day after injection of 0.1 cc of histamine: (*a*) 0.15 molar, +; (*b*) 0.1 molar, +; (*c*) 0.05 molar, +; and (*d*) 0.01 molar, −.

FIG. 9. Necrosis 1 day after injection of 0.1 cc of histamine: (*a*) with concentration much greater than those of Fig. 8, namely, 1 molar, ++, partly fused with necrosis caused by (*b*) 0.15 molar, ++; (*c*) 0.01, +; and (*d*) −.

FIG. 10. Inflammation with no evident skin necrosis 1 day after injection of (*a*) 0.1 cc of 0.1 per cent castor oil, −; (*b*) 0.01 per cent, −; (*c*) 0.001 per cent, −. Necrosis 1 day after injection of 0.1 cc of croton oil: (*d*) 0.01 per cent, ++; (*e*) 0.005 per cent, ++; and (*f*) 0.001 per cent, ++.

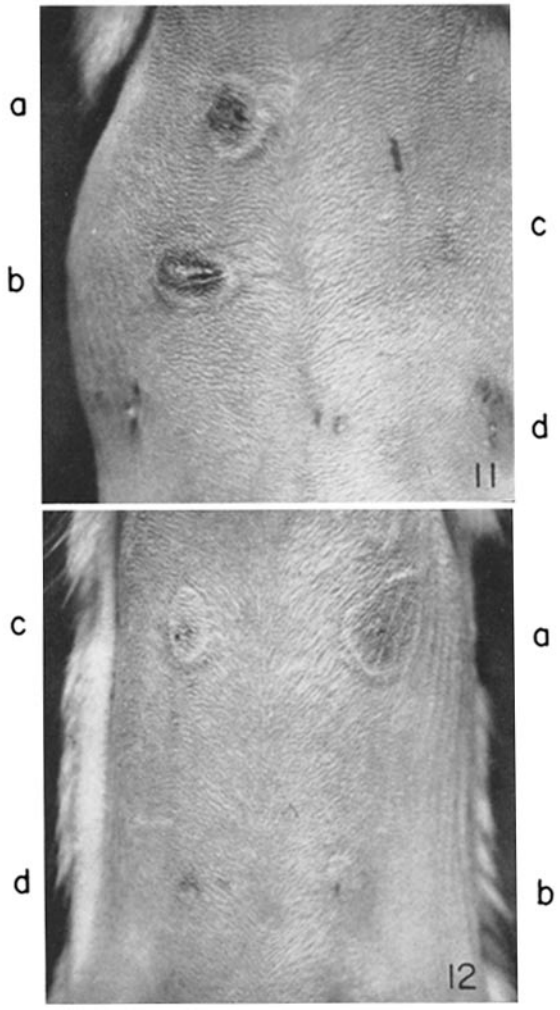


(Opie: Denaturation of proteins)

PLATE 54

FIG. 11. Necrosis 1 day after injection of 0.1 cc of histamine: (*a*) 0.15 molar, ++; (*b*) 0.15 molar (duplicate), ++; (*c*) 0.0015 (for control), −; (*d*) 0.0015 (duplicate), −

FIG. 12. Necrosis 1 day after injection of chloroform with volume injected brought to 0.1 cc by water: (*a*) 0.05 cc, ++; (*b*) 0.02 cc, +; (*c*) 0.01 cc, ++; and (*d*) water (for control).



(Opie: Denaturation of proteins)