served in animals pretreated with either metopirone or Su-9055.

These findings therefore support the suggestion of WONG and WARNER[8] that interruption of the synthetic pathway of adrenal corticoids is not the only way by which DMBA-induced necrosis can be prevented and that competition between Su-9055 and DMBA at the tissue sites is an important factor. However, we should like to modify this hypothesis, and propose that competition for specific receptor sites in the adrenals occurs between the metabolites of DMBA, metopirone and Su-9055 rather than the original compounds. Thus, there is now strong evidence[14,15] that the proximal necrotic agent is the 7-hydroxymethyl derivative of DMBA, and it is also known[16] that metopirone is converted to a secondary alcohol by rat adrenals. In this respect, it is of interest that Su-9055 also contains a keto group which might therefore be reduced in rat tissues[17].

Résumé. Nous avons protegé des rats contre l'action destructive du DMBA sur les adrénales avec des doses de Su-4885 ou de Su-9055 qui n'avaient pas d'effet sur le métabolisme du progestérone et du déoxycorticostérone. Un mécanisme expliquant l'action de ces substances inhibitrices est suggéré.

P. H. JELLINCK[18], M. GARLAND and D. MCRITCHIE

Cancer Research Centre, University of British Columbia, Vancouver (Canada), 17 October 1967.

[14] E. BOYLAND, P. SIMS and C. HUGGINS, Nature *207*, 816 (1965).
[15] D. N. WHEATLEY, A. G. HAMILTON, A. R. CURRIE, E. BOYLAND and P. SIMS, Nature *211*, 1311 (1966).
[16] M. K. BIRMINGHAM and I. KRAULIS, Proc. 2nd Int. Congr. Hormonal Steroids, Excerpta med. *111*, 251 (1966).
[17] We thank Dr. C. W. MURPHY, Ciba Co. Dorval, for a generous gift of metopirone and Su-9055. This work was supported by the Medical Research Council and the National Cancer Institute of Canada.
[18] Present address: Department of Biochemistry, Queen's University, Kingston, Ontario, Canada.

Ascorbic Acid Oxidizes Thiol Groups of Plasma Proteins

Although ascorbic acid usually functions as a reducing agent, it can oxidize thiols such as glutathione[1]. Oxidation of protein thiol groups by ascorbic acid has not been reported. I have observed oxidation of the thiol groups of human plasma proteins by ascorbic acid in vitro at physiological pH.

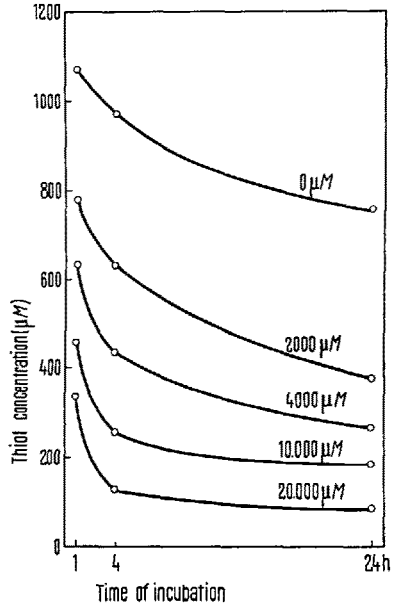
Outdated human blood was obtained from the blood bank, and the plasma was separated by centrifugation. The plasma contained acid citrate dextrose (ACD) anticoagulant.

L-Ascorbic acid was dissolved to make a 0.2 *M* solution in phosphate buffer, and the pH was adjusted to 7.0 with sodium hydroxide solution. An appropriate volume of the ascorbic acid solution was added to 9 ml of plasma, buffer was added to bring the final volume to 10 ml, and the mixture was incubated at 20 °C. Several incubations were done at each concentration of ascorbic acid, with good reproducibility. The same results were obtained when D-araboascorbic acid was used instead of L-ascorbic acid.

Thiol concentrations were measured with 5,5′-dithiobis(2-nitrobenzoic) acid according to the method of ELLMAN[2]. This reagent reacts with both reactive and 'unreactive' thiol groups.

I observed a rapid decrease in the thiol concentration as soon as the ascorbic acid was added. The thiol concentration continued to decrease more slowly for at least 24 h afterwards, as shown in the Figure. This decrease in the thiol concentration is the result of oxidation of thiol groups to disulfide bonds[3]. The rate of oxidation was greater when the concentration of ascorbic acid was increased.

The rapid initial oxidation probably involved the reactive thiol groups, and the slower oxidation that followed probably involved 'unreactive' thiol groups that became accessible to the ascorbic acid as the protein gradually became denatured[3]. A slow oxidation of thiol groups was observed even in the absence of ascorbic acid, as shown by the top curve in the Figure. Because the concentration of glutathione in the plasma is negligible, the major effect of ascorbic acid must be on the protein fraction of the plasma.



Decrease in total thiol concentration of human blood plasma upon incubation with ascorbic acid. The curve labels show the concentrations of ascorbic acid.

[1] P. C. JOCELYN, Biochem. Soc. Symp. *17*, 43 (1959).
[2] G. L. ELLMAN, Archs Biochem. Biophys. *82*, 70 (1959).
[3] R. CECIL, in *The Proteins* (Ed. H. NEURATH; Academic Press, New York-London 1963).

The oxidative ability of ascorbic acid may be important in vivo; for example, in regulating biologically active proteins such as antibodies and enzymes. Additional experiments will be done in this field.

Zusammenfassung. Ascorbinsäure oxydiert die Thiolgruppen der Proteine im Plasma menschlichen Blutes schnell. Diese Fähigkeit der Ascorbinsäure mag für biologische Systeme grössere Bedeutung haben.

J. MEACHAM

Veterans Administration Hospital, Sepulveda (California 91343, USA), 8 September 1967.

Distribution of Inulin-carboxyl-C^{14} in Heart and Skeletal Muscle with Respect to in vivo and in vitro Extracellular Space Determinations

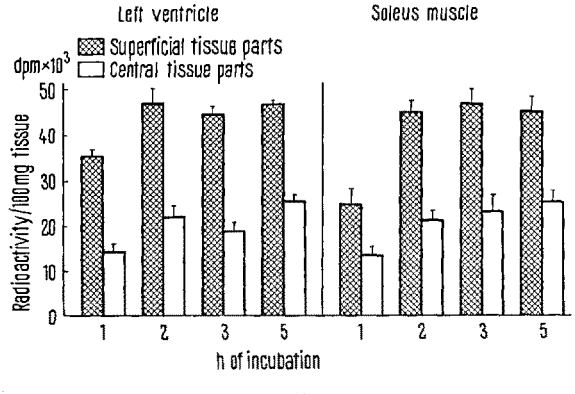
Methods for the determination of extracellular space are based on the same principle, namely the uniform distribution of an indicator throughout the entire extracellular space by the blood stream in vivo and from an incubation fluid in vitro[1]. Inulin is widely used as such an indicator since it is not metabolized and does not penetrate cells as shown by autoradiography[2] and by an osmotic gradient method[3]. In vivo experiments are generally done with nephrectomized animals; equilibrium between the serum and the extracellular space is believed to occur when the serum indicator concentration after i.v. injection falls to a constant level. After the extracellular space values obtained reach a plateau, when plotted against time, it is assumed that an equilibrium in vitro between incubation medium and extracellular space is achieved. This latter equilibration depends on the size and type of tissue as well as the nature of the indicator material and the incubation temperature[4–7].

However, it is not known whether or not these apparent equilibria indeed represent a truly uniform distribution of the indicator material throughout the extracellular compartment. Experiments were designed to verify these assumptions by measuring the distribution of inulin. Tissues exposed to inulin in vivo and in vitro were sectioned into peripheral and central parts and their concentration of radioactivity was compared with each other. With this method a direct estimation of the indicator distribution could be made.

Female golden hamsters of the London School of Hygiene strain inbred at this Institute through over 20 generations, weighing between 100 and 150 g, were used. For the in vivo experiments 8 animals were bilaterally nephrectomized under ether anesthesia and injected in the jugular vein with 0.2 ml (0.68 μc) of 100 mg% solution of inulin-carboxyl-C^{14} (New England Nuclear Corporation, Boston) in phosphate buffer (pH 7.4). Time-course studies demonstrated that the serum inulin level became constant within 60 min. After an additional period of 20 min, a piece of the left ventricle (approximately 100 mg) and the soleus muscle were excised. These tissues were washed free of any superficial radioactive contamination by 6 brief immersions in fresh Ringer's solution, then gently blotted and frozen on dry ice. The frozen tissues were sectioned with a razor blade and divided into 2 groups, a cube-like central part (15–32 mg) and a total of 6 peripheral parts (one for each face of the cube), and the radioactivity content determined separately in the following way: After weighing in tared scintillation vials these tissue pieces were digested with 0.5 ml of a toluene-soluble quarternary ammonium base (NCS, Nuclear Chicago Corporation, Chicago) at 50 °C for 20 h. Fifteen ml of scintillation fluid (4% Liquifluor in toluene, Pilot Chemicals, Watertown, Mass.) were then added to each vial. The radioactivity was measured in a refrigerated scintillation counter (Nuclear Chicago, Model 720), and corrected for background and quenching. In vitro studies were carried out on pieces of left ventricle and intact soleus muscles (cut at the tendon and left attached to the bone). Groups of 6 tissues were incubated for 1, 2, 3 or 5 h at 25 °C in Krebs-Ringer-phosphate buffer, pH 7.1, which contained 25 mg% inulin-carboxyl-C^{14} (85 μc in 100 ml), while being gassed with a mixture of 95% O_2 and 5% CO_2. The distribution of the radioactivity in the center and the surface parts was determined as described above.

The in vivo experiments revealed a nearly uniform distribution of label in the soleus and myocardium. Inside sections of myocardium contained an average of 3% less, those of the soleus muscle an average of 6% more radioactivity than the surrounding tissue. However, in the in vitro experiments no such uniformity was evident. The Figure clearly demonstrates that the central portion of these tissues contained markedly less radioactivity.

Since the pattern of the label distribution is the same in the undamaged soleus as in the myocardial tissue which



Distribution of inulin-carboxyl-C^{14} in tissues incubated in Krebs-Ringer-phosphate buffer (pH 7.1) containing 25 mg% inulin and gassed with a mixture of 95% O_2 and 5% CO_2. Each bar represents the average value of 6 experiments with standard error.

1 P. KRUHØFFER, Acta physiol. scand. *11*, 16 (1945).
2 S. Y. CHOW, W. S. JEE, G. N. TAYLOR and D. M. WOODBURY, Endocrinology *77*, 818 (1965).
3 E. PAGE, J. gen. Physiol. *46*, 201 (1962).
4 H. HOCHREIN, M. REINERT and B. KRIEGSMANN, Z. ges. exp. Med. *139*, 79 (1965).
5 L. E. ROSENBERG, S. J. DOWNING and S. SEGAL, Am. J. Physiol. *202*, 800 (1962).
6 L. BARR and R. L. MALVIN, Am. J. Physiol. *208*, 1042 (1965).
7 E. COTLOVE, Am. J. Physiol. *176*, 396 (1954).