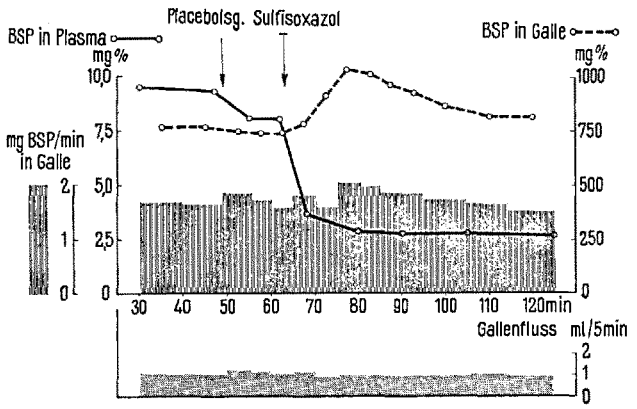
licher Mechanismus wird für die wahrscheinlich ungünstige Wirkung von Sulfonamiden beim Icterus neonatorum gravis in bezug auf das Entstehen eines Kernikterus vermutet[7–9]. Ein vermehrtes Abwandern von BSP ins Gewebe erklärt die vorliegenden Befunde jedoch insofern nicht, als die Sekretion trotz herabgesetzten Blutgehalts mit unverminderter Geschwindigkeit weitergeht. Offensichtlich stellt sich ein neues Gleichgewicht ein mit Erhöhung der BSP-Clearance auf ungefähr das Doppelte der Norm.



Infusion von 0,75 mg/kg/min BSP am Kaninchen. Während das Lösungsmittel von Sulfisoxazol (Placebo) ohne wesentlichen Einfluss bleibt, führt 267 mg/kg Sulfisoxazol i. v. zu einem akuten Abfall der BSP-Blutkonzentration. Die BSP-Sekretion hingegen wird vorübergehend eher noch beschleunigt

Eine Kreislaufwirkung der Sulfonamide von dieser Grössenordnung kommt nicht in Frage. Entweder wird die Farbstoffaufnahme im Bereiche der Leber erleichtert oder der Transport durch die Leberzellen hindurch wird irgendwie beschleunigt. Vermutlich handelt es sich um einen physikalisch-chemischen Vorgang. *In-vitro*-Versuche haben nämlich gezeigt, dass in Gegenwart von Plasma die Aufnahme von BSP durch Leberschnitte verzögert wird[10,11]. In Analogie zum Bilirubin, welches durch Sulfonamide von seiner Eiweissbindung verdrängt wird, kann vermutet werden, dass Sulfonamide auch die BSP-Eiweiss-Bindung irgendwie lockern und damit die Aufnahme in der Leber erleichtern, auch wenn es in den übrigen Gefässgebieten nicht zu einem vermehrten Abwandern ins Gewebe kommt.

Vielleicht besteht zwischen BSP und Bilirubin noch eine weitergehende Ähnlichkeit in dem Sinne, dass auch die Bilirubin-Clearance durch Sulfonamide gesteigert würde. Damit könnten die niedrigen Bilirubinwerte bei Sulfonamid-behandelten Frühgeburten[8] zwanglos erklärt werden. Entsprechende Untersuchungen mit Bilirubin sind im Gange.

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Summary

Investigations in the rabbit demonstrate that even low doses of sulfonamides increase BSP clearance to about twice its normal level.

Antioxidant Activity of Mouse Liver and Kidney Extracts

Antioxidants have been postulated to play a central role in cellular metabolic processes by preventing spontaneous oxidative reactions which may yield products deleterious to the cell[1]. They may be of specific importance in regulating melanin formation by mammalian pigment cells[2,3], and in stabilizing adrenaline within the adrenal medulla[4]. As yet, little is known concerning the chemical nature of tissue antioxidants, particularly with regard to their qualitative and quantitative variation among different mammalian tissues. Several investigators have clearly demonstrated the ability of aqueous extracts of mammalian skin to inhibit the autoxidative conversion of L-3,4-dihydroxyphenylalanine (DOPA) to melanin *in vitro*[2,3,5]. During a recent study designed in part to characterize the chemical nature of inhibitory substances in skin extracts[3,6], parallel examination of mouse liver and kidney extracts was made for comparative purposes. The results are summarized in the present report.

The technics were essentially those reported previously[6]. Briefly, livers and kidneys were removed from adult C3H mice, dissected free of adhering fat, weighed, and suspended in cold double glass-distilled water to yield 50 mg wet weight of tissue/ml. Following homogenization in a chilled Potter-Elvehjem homogenizer, the tissue extracts were stored at – 20°C. Thawed extracts were centrifuged at 12,800 × *g* for 15 min at 0°C, and an aliquot of the supernatant was tested for inhibition of DOPA autoxidation; dry weight of tissue extract was determined for each sample. The test system consisted of 1 ml 5×10^{-3} *M* L-DOPA, 3 ml 0·05 *M* KH_2PO_4-Na_2HPO_4 buffer (pH 7·4), 0·1 ml $1{\cdot}6 \times 10^{-4}$ *M* $CuSO_4$, and water and/or tissue extract to a final volume of 5·1 ml. All reactions were carried out in calibrated test tubes, which were incubated at 37°C. Progress of melanin formation was followed by taking hourly readings on a Klett-Summerson colorimeter with a KS-42 (blue) filter. The percent inhibition of DOPA autoxidation by tissue extracts was calculated as a function of experimental and control values at the fourth hour of incubation. Precipitation of proteins was performed by adding 5 *M* trichloroacetic acid (TCA) to tissue extracts (final concentration TCA = 0·8 *M*). Following centrifugation, the precipitate was dissolved in a volume of 0·1 *N* NaOH such that the pH did not exceed 9·0; the resulting mixture was neutralized with HCl. Neutralization of the supernatant was accomplished with NaOH. The fractions thus obtained were tested for action on DOPA autoxidation. Tissue extracts were also tested for heat stability, dialyzability, and reversibility of inhibitory activity by sulfhydryl blocking agents[6].

In confirmation of Hirsch[7], liver and kidney extracts produced a marked inhibition of DOPA autoxidation. Liver yielded 9·1 ± 0·3 mg dry weight of extract from 50 mg wet weight, and the percentage inhibition of DOPA autoxidation was 56·4 ± 3·7 per 0·5 mg dry weight of extract (means ± mean S. E. based on 6 determinations).

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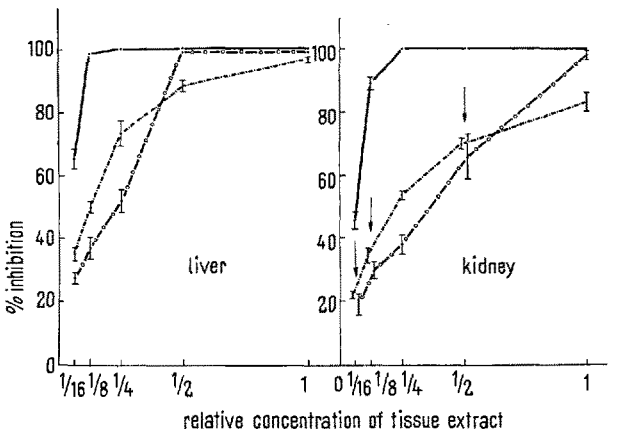
Corresponding determinations for kidney were $6{\cdot}1 \pm 1{\cdot}8$ and $57{\cdot}3 \pm 1{\cdot}8$, respectively. Expressed on the same basis, inhibition by mouse diaphragm was 28%, and by human and mouse skin 18%[6]. The inhibition by liver and kidney extracts was partly reversible by the addition of sulfhydryl blocking agents (Fig.). The maximum reduction of inhibition by sulfhydryl reagents was 60–70% for both liver and kidney. Our recent preliminary estimations of water-extractable -SH derived from 50 mg wet weight of tissue yielded values of 0·25 μM for liver and 0·18 μM for kidney[8]. A previous study of inhibition of DOPA autoxidation by liver and kidney extracts failed to demonstrate an -SH component of the inhibitor[7]; similarly, manometric studies of antioxidant activity of plasma proteins[9] and adrenal medulla[4] failed to reveal an -SH factor. The demonstration of an active sulfhydryl fraction in the present study might be the result of the differences in method and/or the establishment of a correct molar relationship between inhibitor and -SH reagents (Fig.). It is noteworthy that corrections had to be made throughout for the effect of -SH blocking agents on the DOPA system; both iodoacetamide and iodosobenzoate tended to accelerate DOPA autoxidation, whereas *p*-chloromercuribenzoate inhibited the process.

A loss in inhibitory activity of 40–60% occurred when liver and kidney extracts were placed in a boiling water bath for 20 min. The inhibitory fraction which survived boiling was almost entirely non-reversible by sulfhydryl reagents. These findings suggest that aqueous liver and kidney extracts, like those of skin and diaphragm[6], contain a complex of antioxidants consisting of at least two major fractions; one, heat-labile and -SH in nature; a second, non-SH and heat-stable. The bulk of inhibitory substances in liver and kidney extracts remained undialyzed after 48 h dialysis against distilled water in the cold. Control liver extracts produced a 52% inhibition of DOPA autoxidation per 0·5 mg dry weight of tissue extract, whereas dialyzed extracts inhibited 45%; similar values for kidney extracts were 60% and 45%, respectively.

Treatment of liver and kidney extracts with TCA suggested that the inhibitory complex was almost entirely proteinaceous in nature. Whereas 1 ml of liver or kidney extract inhibited DOPA autoxidation 100% (Fig.), the supernatants of both organ extracts following treatment with TCA (4 determinations each) inhibited less than 8%, and in half the cases enhanced DOPA autoxidation by about 10%. Conversely, the resuspended protein precipitates from 1 ml of liver or kidney extract inhibited 93% and 50%, respectively. Addition of iodosobenzoate to the resuspended precipitates lowered their inhibitory activity by 47% for liver and 67% for kidney, strongly suggestive of an involvement of protein -SH as part of the inhibitory complex.

A role of protein -SH in the inhibition of DOPA autoxidation was further supported by experiments with crystalline bovine serum albumin (BSA). This sulfhydryl-containing protein, when added to the test system in amounts of 0·33 to 33 mg, produced a marked inhibition of DOPA autoxidation. However, the effect of BSA on the DOPA system was difficult to interpret, for the inhibition was found to decrease with increasing concentration of BSA (inhibition by BSA: 0·33 mg = 47%; 8·2 mg = 63%; 33 mg = 21%; 66 mg = – 12%). As indicated, the addition of sufficient amounts of BSA (66 mg) to the test system actually enhanced DOPA autoxidation. Iodosobenzoate brought about a reduction of inhibition which increased from 30% to 60% with decreasing concentration of BSA. Although the effect of BSA on DOPA autoxidation is obviously quite complex[10,11], it may be concluded that pure proteins under specific conditions can act as antioxidants, and that part of this activity is attributable to protein -SH.

It would appear, therefore, that antioxidants capable of inhibiting autoxidation of DOPA *in vitro* are widely distributed among mammalian tissues and are at least partly proteinaceous in nature[1]. Our findings suggest that protein -SH may be of greater importance in antioxidant activity of tissue extracts than heretofore thought[1]. Only fragmentary evidence is available concerning the functions of tissue antioxidants in metabolic processes of living cells. It is clear that various organs contain different amounts of these substances, and it may be of significance that tumor tissues from mice[1] and blood plasma from human cancer patients[12] are deficient in antioxidants. The postulated role of antioxidants in melanin formation by mammalian melanocytes[2,3] and in the stabilization of adrenaline within the adrenal medulla[4] may well represent specific functions of a more general system of biological control. However, additional work is necessary to critically test HIRSCH's interesting suggestion that antioxidants are part of a mechanism of cellular stabilization protecting the cell against spontaneous formation of free radicals, mutagens, carcinogens, and other deleterious substances[1].



Effect of sulfhydryl reagents on inhibition of DOPA autoxidation by liver and kidney extracts. Mean values of inhibition are plotted together with mean standard errors based on 9, 5, and 4 determinations, respectively, for tissue extract (·—·), tissue extract +0·1 mg iodosobenzoic acid (·–·–·), and tissue extract + 0·1 mg *p*-chloromercuribenzoic acid (›—‹—‹). A maximum of 1 ml of tissue extract was added to the test system. Several points (arrows) have been shifted slightly to permit clear illustration of data

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Biological Station, Roswell Park Memorial Institute, Springville (N. Y.), December 7, 1959.

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Zusammenfassung

Die hemmende Wirkung wässeriger Leber- und Nierenextrakte von Mäusen auf die DOPA-Auto-Oxydation wurde geprüft. Leber- und Nierenwerte waren von gleicher Grössenordnung und etwa dreimal grösser als die früher für Mäuse- und Menschenhaut bestimmten Werte. Der Hemmfaktor besteht zum grossen Teil aus Eiweiss und konnte in einen -SH-Gruppen enthaltenden, hitzeempfindlichen Teil und einen -SH-Gruppen-freien, hitzestabilen Teil getrennt werden. Die mögliche Bedeutung solcher Hemmstoffe für die Zelle wird erörtert.

On the Elimination of Chlorprothixene in Rat and Man

Chlorprothixene[1], the trans isomer of 2-chloro-9-(3-dimethylaminopropylidene)-thiaxanthene, hydrochloride, is a new tranquillizing drug, the pharmacological properties of which have been reported previously[2,3] and which has been found to have promising clinical properties[4–7]. It is an analogue to chlorpromazine in which the phenothiazine ring system has been replaced by the thiaxanthene ring system to which the side chain is attached by a double bond. Like chlorpromazine it shows a fairly high ultraviolet absorbancy. UV maxima are obtained at 230, 268, and 325 mμ in aqueous solution. It is easily extracted from an alkaline aqueous solution into nonpolar solvents such as heptane or ether, and from these solvents into dilute acid. On addition of an equal volume of concentrated sulfuric acid to an aqueous solution, a weak salmon pink colour is obtained. The solution shows a main absorbance maximum at 389 mμ and additional maxima at 492 and 518 mμ. Furthermore, the solution shows intense yellow fluorescence (maximum at 559 mμ) on irradiation with UV light (around 3000–4000 Å). All these properties may be used for analysis qualitatively and quantitatively.

On analysis of urine from rats given the drug parenterally or orally, and from patients taking the drug orally, a sulfuric acid reaction as given above was found with extracts. The UV absorbance curves were not the same as obtained for the drug, however. Thus the presence of a metabolite was suspected and this was suggested to be the corresponding sulfoxide, in analogy to the case with a number of phenothiazine tranquillizers[8–11]. Synthetic chlorprothixene sulfoxide showed UV absorbance maxima at 221 and 261 mμ and a 'shoulder' at 310 mμ, and the UV absorbance curve was identical with the curves obtained for the urine extracts. On carrying out the sulfuric acid reaction, a salmon pink colour was obtained and the absorbance curve showed maxima at about 390 and 500 mμ; which is the same finding as for the urine extracts. No fluorescence in UV light was obtained for the synthetic sulfoxide or the urine extracts. On paper-chromatography in butanol-acetic acid-water, urine extracts gave a UV absorbing spot in the same position as the synthetic sulfoxide, R_f about 0·70, whereas the unchanged drug gave R_f about 0·75 and showed yellow fluorescence in long wave UV light after treatment with 50% sulfuric acid. No such reaction was obtained for the sulfoxide or the urine extracts.

After elution of the spot obtained with urine in dilute acid, a UV absorbance curve identical to that of the sulfoxide was obtained. On paper chromatography in 0·067 *M* phosphate buffer[12], pH 7·5, the sulfoxide and the urine extracts gave R_f about 0·70 and the drug R_f about 0·10. The identity of the urinary metabolite with the sulfoxide was thus concluded.

On quantitative analysis of the output of sulfoxide in urine (modified extraction according to SALZMAN and BRODIE[8] for separation of unchanged drug and sulfoxide; acetate buffer pH 5·4 was used), up to about 5% of the dose was recovered in 48 h on oral administration to rats (single dose, 25 mg/kg). In man 5·9–29·0%, with a mean of 11.7%, was recovered in 24 h (on continued administration with doses up to 100 mg thrice daily).

On analysis of feces (same method as for urine) unchanged drug as well as the sulfoxide was found, on oral administration in man. The total elimination in feces varied from 0–41% of the dose, somewhat more of the unchanged drug being eliminated. No other metabolites have been identified so far. In rats, only the sulfoxide metabolite was found in feces, and in amounts from 1–7% of the dose in 48–72 h on both oral and parenteral administration (single dose).

In the bile from bile-fistula-rats (light urethane-ether anesthesia), only sulfoxide was found and in amounts of the same order as in feces, after oral or intramuscular administration. After intravenous injection, however, up to 24% of the dose was recovered as sulfoxide from the bile within 7 h.

No detectable amounts of drug or sulfoxide were found in blood on oral administration to man, nor could the drug be determined in blood following oral or intramuscular administration of 25 mg/kg or intravenous injection of 5–10 mg/kg to rats. 2–5 min after intravenous injection of 25 mg/kg, a blood concentration in the order of 3–1 μg/ml was observed. The drug had disappeared from the blood in less than 10 min after injection.

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Zusammenfassung

Die Ausscheidung des neuen Neuroleptikums, Chlorprothixen, bei Menschen und Ratten wurde in Urin, Galle und Faeces untersucht und als einziges Abbauprodukt Chlorprothixen-Sulfoxyd gefunden. Ausscheidungsprodukte in % der verabreichten Dosis: Beim Menschen (orale Verabreichung) im Urin: 5–29% Sulfoxyd, Faeces: 0–41% unveränderter Stoff + Sulfoxyd. Bei der Ratte (orale oder parenterale Verabreichung) im Urin: bis zu 5% Sulfoxyd, Faeces: 1–7% Sulfoxyd, keine unveränderte Substanz, Galle: bis zu 24% Sulfoxyd.

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