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Compounds of Quinone Structure as Allergens and Cancerogenic Agents

By R. L. MAYER[1], Summit, N. J.

1. *Introduction*

The widespread use of azo-dyes as coloring agents for fabrics, cosmetics, and foods has recently been the subject of discussions regarding their innocuousness. BAER, MELTZER and MAYER[2], and MAYER[3] have drawn attention to the possibility that azo-dyes as food colors may be the cause of certain persistent allergic skin reactions of unknown etiology and BAUER and KUHN[4] have discussed the major role that these azo-dyes may play in the increased incidence of cancer.

Certain azo-dyes and closely related aromatic amines and nitro compounds are powerful antigens and frequently cause a variety of persistent allergic reactions such as dermatitis, urticaria, and asthma. Of almost all the allergies of known etiology, those produced by these compounds have a great tendency towards unexpected recurrences, which may be explained by the fact that they comprise a number of cross-sensitizations long considered as independent allergies, each of which is produced by a variety of substances of common use. Similarly conspicuous are the specific toxic manifestations produced by aromatic amines and nitro compounds, especially methemoglobinemia and disturbances in the central nervous system, which in certain instances constitute dangerous occupational hazards.

In addition to the various forms of allergic manifestations and intoxications which they engender, azo-dyes, aromatic amines and nitro compounds are capable of producing atypical cell proliferations and cancer, generally referred to as "aniline cancer" or "butter yellow cancer". From the pathogenetic point of view, this cytological reaction is apparently different from the allergic and purely toxic reactions.

In this review the conditions under which the allergic and cytological reactions occur after contact with these aromatic compounds will be discussed. Since the author believes, that as in the case of intoxications with aromatic amines or nitro compounds, allergic and cytologic reactions are produced not by the unaltered chemicals but by metabolites, certain metabolic transformations which precede the outbreak of the various pathologic manifestation will be considered.

Until now, the formation of methemoglobin brought about by amino and nitro derivatives has been the most generally accepted indicator for their metabolic transformation into certain oxidation or reduction products and intermediates between amines and nitro compounds. But it has been impossible to decide which of the various possible intermediates was most likely to occur or which was instrumental in the formation of methemoglobin. Indeed, the intermediate metabolic transformation products of aromatic amines and nitro compounds are unstable for they have not as yet been isolated or identified with sufficient certitude. In the course of the studies reviewed in this article it became apparent that not only methemoglobinemia, but also allergies and cytological changes may constitute additional useful tools for identifying the intermediates. In fact, allergies and cell proliferations seem to be even more specific reactions than the development of methemoglobinemia.

2. *Allergic Hypersensitivity to aromatic amines and nitro compounds*

Many azo-dyes, aromatic amines and nitro compounds are most powerful sensitizers (MAYER[5], SCHWARTZ[6]). The most common symptoms are allergies of the skin, especially contact dermatitis and the so-called neurodermatitis; a less frequent form is urticaria. Certain amines produce asthma, intestinal symptoms and combinations of these manifestations. Of all the amines, p-phenylenediamine (PPD), technically known as "Ursol", is not only the strongest, but also the most versatile sensitizer; it is one of those very rare antigens capable of producing almost any type of allergy from dermatitis to asthma. Ursol allergy has therefore been intensively studied, clinically as well as experimentally, and Ursol dermatitis was among the first cutaneous

[1] Research Laboratories, Ciba Pharmaceutical Products, Inc., 556 Morris Avenue, Summit, N. J.

[2] R. L. BAER, L. MELTZER, and R. L. MAYER, Proc. Soc. Exp. Biol. and Med. *67*, 489 (1949).

[3] R. L. MAYER, J. Allergy *20*, 159 (1949).

[4] K. H. BAUER, Dtsch. Chirurgenkongreß 6. 8. 1949, Frankfurt.

[5] R. L. MAYER, Arch. Dermatol. Syph. *163*, 223 (1931).

[6] L. SCHWARTZ, L. TULIPAN, and S. M. PECK, *Occupational Diseases of the Skin* (Philadelphia, 1947).

allergies to be produced experimentally in guinea pigs (MAYER[7, 8]). Allergy produced by PPD regularly crosses over to that induced by a number of other aromatic amines chemically related to PPD.

3. *The various groups of allergies participating in cross-sensitizations to aromatic amines*

The extension of the cross-sensitization under discussion was established by various steps. The first known cases of such cross-sensitization proved to be sensitive simultaneously to certain aromatic amines and azo-dyes (MAYER[8]) and had acquired an allergy by contact with PPD, p-aminophenol, 1–4-diaminophenol, aniline and fabrics or furs treated with these substances, or had been sensitized initially to certain azo-dyes such as aminoazobenzene, aminoazotoluene and their acetyl and diacetyl derivatives used for dyeing fabrics, leather or cosmetic preparations, or intended as therapeutic agents.

A second type of cross-sensitivity to aromatic amines was first described in 1936 by FLANDIN and co-workers[9] and later by TZANCK[10] and SIDI[11], who encountered patients simultaneously sensitive to PPD, aniline and local anesthetics derived from p-aminobenzoic acid (PABA), such as procaine, orthoform and others. Most of these allergies were acquired by sensitization through the use of certain local anesthetics contained in therapeutic ointments; in rarer instances by contact with furs or hair dyed with PPD.

In a third type of cross-sensitization the patient is hypersensitive simultaneously to local anesthetics of the procaine group and to sulfonamides (PHILLIPS[12], SULZBERGER and co-workers[13] and ROGERS[14]).

The fourth and last type of cross-sensitization comprises cases simultaneously sensitive to aromatic amines and nitro compounds, as reported by TZANCK[10] and SIDI[11].

Only one case has been described which broadly embraces all the above-mentioned types and which can be considered as the prototype of this cross-sensitivity: a patient of MELTZER'S and BAER'S[15] who was specifically hypersensitive to PPD, aniline, azo-dyes, sulfonamides, picric acid, PABA and related local anesthetics (see Fig. 1). The fact that hypersensitivity against the entire group of substances has been detected in only one patient is no proof of the rarity of such cases. It is, on the contrary, very probable

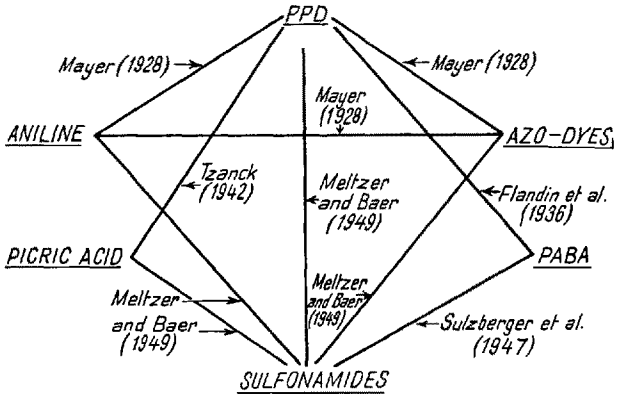


Fig. 1. – Cross-sensitization between aromatic amines, azo-dyes, nitro compounds, sulfonamides, and PABA.

that many of the previously examined cases possessed a broader allergic spectrum than that disclosed by the limited number of tests made.

4. *Substances participating in sensitizations to aromatic amines and azo-dyes*

It has been conclusively shown in the analysis of the various cases that the following substances participate in the cross-sensitization under consideration: (a) aromatic amines, aminophenols, diaminophenols and diamines; (b) azo-dyes containing free or substituted aromatic amino groups; (c) certain aromatic mono- and poly-nitro derivatives and nitrophenols; (d) sulfanilamide and substituted sulfonamides; (e) PABA and its esters. But not all amines and azo-dyes containing free or substituted amino groups proved to be allergenic to the same extent. There is a long series of amines ranging from those which possess both high sensitizing and eliciting powers, to others which lack sensitizing power but are nevertheless elicitors (MAYER[16]) and finally to amines that are allergenically inactive, devoid of both sensitizing and eliciting activity (CALVERY[17]). The most important of the allergenic amines are listed in Tables I, II, and III, which also contain examples of related non-allergenic substances.

All these substances share the following outstanding properties: (1) They contain aromatic amino groups which are readily oxidized, or nitro groups which are easily reduced; it has been established that such substances are metabolized within the organism and excreted to a variable extent, in a chemically altered form. (2) All active substances have low molecular weights and are therefore, from the immunological

[7] R. L. MAYER, Arch. Dermatol. Syph. *163*, 223 (1931).
[8] R. L. MAYER, Arch. Dermatol. Syph. *156*, 331 (1928); Klin. Wschr., 2. Hälfte, 1958 (1928); Arch. Dermatol. Syph. *158*, 266 (1939).
[9] C. FLANDIN, H. RABEAU, and Mlle UKRAINCZYK, Anesth. Analg. *3*, 102 (1937); Bull. Soc. franç. Dermat. Syph. *45*, 1869 (1938).
[10] B. H. TZANCK, *Accidents cutanés etc.* (Arnette, Paris 1945).
[11] E. SIDI, *Les accidents cutanés etc.* (Médicales Flammarion, Paris 1945).
[12] B. PHILLIPS, Brit. J. Dermat. *58*, 213 (1946).
[13] M. B. SULZBERGER, A. KANOF, and R. L. BAER, J. Allergy *18*, 92 (1947).
[14] E. B. ROGERS, J.A.M.A. *111*, 2290 (1938).
[15] L. MELTZER and R. L. BAER, J. Invest. Dermat. *12*, 31 (1949).
[16] R. L. MAYER, J. Allergy *20*, 159 (1949).
[17] H. O. CALVERY, Amer. J. Pharm. *114*, No. 9 (1942).

Table II
Azo-dyes

A		B	
Allergenic	Reference	Non-allergenic	Reference
Diazoaminobenzene	4	Benzol-azo-diphenylamine	4
Azoxybenzene	4	Benzol-azo-resorcine	4
Aminoazobenzene	4	2,4-4'-Triaminoazobenzene	4
Methylaminoazobenzene	4	2,4-3'-Triamino-5'-methylazobenzene	4
Dimethylaminoazobenzene	4	4-Nitro-4'-diethylaminoazobenzene	12
Methyl-orange	4		
Amino-azotoluene	4		
Acetylamino-azotoluene	4		
Diacetylamino-azotoluene	4		
3-Aminobenzene-azo-m-toluidine	4		
1-Phenylazo-2-naphthylamine	4		
4-Acetylaminobenzene-azo-chlorophenol	12		

standpoint, "haptens" and thus unable to sensitize as such. Their antigenicity depends, according to the generally accepted theory, upon their ability to form complexes with "carriers" of high molecular weight (LANDSTEINER[18]).

Table I
Aromatic amines and nitro compounds

A		B	
Allergenic	Reference	Non-allergenic	Reference
Aniline	4, 6, 8, 12	m-Phenylene-diamine	4
o-Phenylenediamine	4	2,4-Diaminotoluene	4
p-Phenylenediamine	4	2,6-Diaminophenol	4
o-Aminophenol	4	p-Nitrobenzoic acid	12
p-Aminophenol	4	3,5-Dinitrobenzoic acid	12
1,4-Diaminophenol	4		
2,4-Diaminophenol	4		
2,5-Diaminophenol	4		
Nitrobenzene	4, 8, 9, 12		
Picric acid	4, 8, 9, 12		

The inclusion of the various chemical substances such as amines, nitro compounds, sulfonamides and aminobenzoic acid into a single allergic entity can only be explained on the basis that they share a common immunologic denominator. It is evident that this denominator is either the common amino group—preformed or derived from nitro compounds by metabolic reduction—or an intermediate metabolite which is not an amine.

Table III
Sulfonamides and esters of PABA-producing cross-sensitivity

A. Sulfonamides		B. p-Aminobenzoic acid	
Substances	Reference	Substances	Reference
Sulfanilamide	9, 10, 11, 12	PABA	12
Sulfaguanidine	9, 10, 11, 12	Procaine	8, 9, 10, 12
Sulfadiazine	9, 10, 11, 12		
Sulfapyridine	9, 10, 11, 12	Benzocaine	12
Saccharine	12	Butesine	12

[18] K. LANDSTEINER, *The Specificity of Serological Reactions* (Cambridge, 1945).

In a search for the common immunologic property among the different substances it is therefore necessary to analyze: (a) the specific antigenic character of an aromatic amino or nitro group, (b) the antigenic character of the various metabolic transformation products formed from substances containing such groups, and (c) the affinities of the amino or nitro groups and their metabolites for carrier substances.

5. *The chemical groups responsible for the cross-sensitizations*

Certain substances included in this allergy are "immunologically monovalent". They contain only one active group which provokes the formation of specific antibodies; such substances are: aromatic amino or amino-oxy groups as in amines, aminophenols, diamines, diaminophenols, azo-dyes, sulfonamides, and aromatic nitro groups. In these instances the immunological monovalence can be so specific that hypersensitivity is restricted to the sensitizing agent, or it only extends to stereo-isomers, optical isomers and other chemically related compounds (LANDSTEINER and co-workers[19]).

In other instances the antigens contain multiple immunologically active groups, as for instance in the case of local anesthetics derived from PABA, which are composed of at least three or four immunologically active centers (ROTHMAN *et al.*[20], SCHWARZSCHILD[21], LADEN and RUBIN[22]). It has been shown that only the hypersensitivity produced by the aminobenzoic acid nucleus of local anesthetics participates in a cross-sensitization to aromatic amines.

[19] K. LANDSTEINER, *The Specificity of Serological Reactions* (Cambridge, 1945).
[20] S. ROTHMAN, F. J. ORLAND, and P. J. FLESCH, Invest. Dermat. *6*, 191 (1945).
[21] L. SCHWARZSCHILD, Arch. Dermat. und Syph. *156*, 432 (1925).
[22] E. L. LADEN and L. RUBIN, Proc. Soc. Exp. Biol. Med. *66*, 451 (1947).

6. *Mechanism of action*

I. Metabolic transformations of the antigens

As has been pointed out above, one may assume that the immunologically active component of the molecules of aromatic amines, azo-dyes, sulfonamides and PABA capable of producing the same type of allergic sensitization, is represented either by a common, preformed chemical group or by a common metabolite.

Since the various substances involved in this cross-sensitivity are aromatic amines or nitro compounds which are readily reduced to amines, or azo-dyes yielding aromatic amines upon reduction, many investigators have considered the aromatic amino group, especially a primary amino group in para-position to the other substituents, as the actual offender, and have referred to this allergy as "a cross-sensitization to primary aromatic amino groups" (FLANDIN and co-workers[23], TZANCK[24], SIDI[25], NITTI and co-workers[26], BAER[27]).

If this explanation were correct, then all or the majority of aromatic amines, particularly compounds having an aromatic amine in the para-position, should participate in this form of allergy. But tests with a great variety of amines and azo-dyes have shown that numerous substances do not elicit allergic reactions in patients sensitive to PPD, aniline, or azo-dyes (see Tables I, II, and III). The conclusion must therefore be drawn that the unaltered primary amino group as such cannot be the directly responsible factor in this sensitization. Furthermore, such an explanation would not necessarily explain the inclusion of certain nitro compounds in this type of cross-sensitization.

The second approach in quest of a common immunological property is the consideration of an intermediate metabolic transformation of the original antigen.

The animal organism can attack chemically all substances involved in this cross-sensitization and excrete them (I) coupled to an organic or inorganic acid or (II) in either an oxidized or reduced form. *Aromatic amines* undergo two major metabolic transformations: (1) coupling with acids to form acetyl derivatives, ethereal sulfates or glucuronides, and (2) oxidation (STEVENSON *et al.*[28]; lit. see WILLIAMS[29]).

Whereas acetylation and the formation of glucuronides and ethereal sulfates lead to a detoxification of otherwise toxic substances, the oxidation of aromatic amines, on the contrary, is the prototype of an autointoxication.

Closely related to the oxidation of aromatic amines is the reduction of *aromatic nitro compounds* to amines and oxyamines (MEYER[30], NEUBERG[31] *et al.*, CHANNON *et al.*[32], LANDSTEINER and DI SOMMA[33]; lit. see WILLIAMS[34]).

The oxidation of aromatic amines and the reduction of nitro compounds probably occur in several steps. Possible intermediates are phenylhydroxylamines, nitroso compounds, compounds of quinone structure and azoxy derivatives. The formation of methemoglobin by aromatic amines and nitro compounds is explained by HEUBNER et al.[35], ELLINGER[36] and WILLIAMS[34] on the basis of the high oxidizing power of these intermediate oxidation products; but it is almost impossible to decide whether the principal intermediate is a nitroso derivative, a hydroxylamine, a quinone, or a quinone imine. In accordance with this view, RIMINGTON and HEMMINGS[37] formulate the general hypothesis that the chemical grouping necessary for methemoglobin formation is an aromatic amino group, unsubstituted or potentially free, which is capable of undergoing oxidation with the resulting formation of a hydroxylamine derivative or a reversible oxidizing system such as p- or o-quinone imine. By actual chemical isolation ELLINGER[36] has identified tolyl hydroxylamine as a metabolite of aminotoluene, but WILLIAMS[34] has questioned the correctness of this identification. Since no phenylhydroxylamine as such appears in the urine, it is probable that if it is formed it is rearranged to aminophenol before excretion.

Oxidation of ingested PPD by living cells seemingly does not produce a hydroxylamine. According to ERDMANN and VAHLEN[38], and HEUBNER and MEIER[35], it is oxidized directly into quinone diimine, which in turn produces the methemoglobinemia.

The biological degradation of *azo-dyes* is more complicated. Living cells are capable of reducing these substances, using the –N=N– bond as a hydrogen acceptor and thus splitting the molecule to furnish two aromatic amines. Besides the reductive breakdown of the azo linkage, other metabolic modifications of the azo compound are conceivable, such as formation of hydrazobenzene or oxyazobenzene.

[23] C. FLANDIN, H. RABEAU, and Mlle UKRAINCZYK, Anesth. et Analg. *3*, 102 (1937); Bull. Soc. franç. Dermat. Syph. *45*, 1869 (1938).
[24] B. H. TZANCK, *Accidents cutanés etc.* (Arnette, Paris 1945).
[25] E. SIDI, *Les accidents cutanés etc.* (Médicales Flammarion, Paris 1945).
[26] F. NITTI, D. BOVET, and F. DEPIERRE, Rev. Immunol. *3*, 376 (1937).
[27] R. L. BAER, Arch. Dermat. Syph. *58*, 276 (1948).
[28] E. S. STEVENSON, K. DOBRINER, and C. P. RHOADS, Cancer Research *2*, 160 (1942).
[29] T. WILLIAMS, *Detoxication Mechanisms* (London, 1947).
[30] E. MEYER, Z. Physiol. Chem. *46*, 497 (1905).
[31] C. NEUBERG and E. WELDE, Biochem. Z. *67*, 18 (1914).
[32] H. J. CHANNON, G. T. MILLS, and R. T. WILLIAMS, Biochem. J. *38*, 70 (1944).
[33] K. LANDSTEINER and A. A. DI SOMMA, J. Exper. Med. *72*, 361 (1940).
[34] T. WILLIAMS, *Detoxication Mechanisms* (London, 1947).
[35] W. HEUBNER, Arch. exper. Path. Pharmakol. *72*, 239 (1929). – W. HEUBNER and H. RHODE, Arch. exper. Path. Pharmakol. *100*, 117 (1923). – W. HEUBNER and R. MEIER, Arch. exper. Path. Pharmakol. *100*, 137 (1923).
[36] P. ELLINGER, Z. physiol. Chem. *111*, 186 (1920).
[37] C. RIMINGTON and A. W. HEMMINGS, Biochem. J. *33*, 960 (1939).
[38] E. ERDMANN and E. VAHLEN, Arch. exper. Path. Pharmakol. *53*, 401 (1906).

The mechanism of the metabolic splitting of the –N=N– group was first described by the author in 1928 (MAYER[39]), in the case of aminoazobenzene.

In 1936 TRÉFOUËL and co-workers[40] described the same reaction in the case of Prontosil and thus discovered the chemotherapeutic action of the liberated sulfanilamide. STEVENSON and co-workers[41] in 1942 detected PPD in the urine after feeding butter yellow.

The metabolic transformation of *sulfonamides* is as yet little understood. As in the case of other amines, acetylation of variable extent is the most common and regular metabolic change.

When in 1937 the author formulated the theory that sulfanilamide undergoes metabolic oxidation to hydroxylamino-sulfanilamide (MAYER[42], MAYER and OECHSLIN[43], MAYER[44]), many discussions arose regarding the possible metabolic oxidation of sulfonamides and the role of their oxidation products with respect to toxicity and activity. It was indeed postulated that these oxidation products were responsible for methemoglobinemia.

Various objections to the hydroxylamine theory have been formulated. WILLIAMS[45] has isolated 3-hydroxy sulfonamides from urine after ingestion of sulfanilamide and substituted products such as sulfapyridine and sulfathiazole and considers 3-hydroxy sulfonamide as the actual and only oxidation product of sulfonamides. This view does not seem to be correct. Just as aminophenols in their unchanged chemical state are unable to form methemoglobin, 3-hydroxy sulfonamide similarly cannot produce methemoglobinemia without previous oxidation. It may thus be concluded that, as in the case of the other aromatic amines, the formation of an intermediate labile oxidation product from sulfanilamide is inescapable; the detection of 3-hydroxy sulfonamide in the urine after ingestion of sulfonamide may well constitute proof in favor of the hydroxylamine theory of the sulfonamide oxidation (see also ROSENTHAL and BAUER[46]). WOODS'S[47] theory seemed to replace the hydroxylamine theory with a more suitable explanation regarding the mechanism of action of sulfonamides. The question arises whether the "hydroxylamine theory" of sulfonamides is incompatible with WOODS's theory. In our opinion, as well as in that of DANN and MÖLLER'S[48], it is conceivable that the metabolically formed oxidation products of sulfonamides and PABA, rather than the original amino compounds, compete with each other.

The very fact that sulfonamides participate as active members in a cross-sensitization in which (I) the other participants are PPD and other amines, nitro compounds, aminobenzoic acid, etc. and in which (II) the active antigen is a metabolite formed from PPD and other amines by partial oxidation or from nitro compounds by partial reduction, adds considerable weight to the "oxidation theory" of sulfonamides.

Very little is known regarding the metabolic changes of PABA and its esters. A considerable portion is excreted after acetylation or after formation of glucuronides upon previous oxidation of the acid or the hydrolysis of its esters. However, the fact that PABA participates on the same level as sulfonamides, PPD and other amines in an allergic cross-reaction indicates that its metabolic transformations are similar to those which were discussed in the case of sulfonamides.

In recent studies on the biological transformation of PABA by microorganisms, yellow pigments formed from PABA and its esters and from p-aminosalicylic acid (PAS) by actively growing mycobacteria were observed which very likely are N-oxidation products of PABA and PAS (MAYER[49]). In unpublished experiments, SLOANE and MAYER were able to demonstrate the occurrence of aniline and aminophenol during this reaction.

7. *Mechanism of action*

II. The possible role of hydroxylamines and quinone compounds in the immunologic process

We are thus faced with two different metabolic reactions by which an immunologically identical intermediate can be formed from aromatic amines, azodyes, and nitro derivatives and which may explain their common sensitizing power: either (1) coupling with acids, namely formation of acetyl derivatives, glucuronides, ethereal sulfates, or (2) the formation of various oxidation and reduction products. Which of these metabolites constitutes the direct antigen?

Whether a substance can be considered as the causative agent for a given allergy is determined either by serological tests (LANDSTEINER[50]) or, in the case of the contact dermatitis type, by direct skin test. In this case the chemical, applied in appropriate concentrations to the skin of an allergic animal or individual, will produce an inflammation if it constitutes the sensitizing agent. This test was employed in the following experiments.

[39] R. L. MAYER, Arch. Dermatol. Syph. *156*, 331 (1928).

[40] J. TRÉFOUËL, Mme J. TRÉFOUËL, F. NITTI, and D. BOVET, C. R. Soc. Biol. *120*, 756 (1936).

[41] E. S. STEVENSON, K. DOBRINER, and C. P. RHOADS, Cancer Research *2*, 160 (1942).

[42] R. L. MAYER, Bull. Acad. Méd., Paris *117*, 727 (1937).

[43] R. L. MAYER and C. OECHSLIN, C. R. Soc. Biol. *130*, 211 (1939).

[44] R. L. MAYER, C. R. Soc. Biol. *130*, 1560, 1562 (1939).

[45] T. WILLIAMS, Biochem. J. *35*, 1169 (1941).

[46] S. M. ROSENTHAL and H. BAUER, Public Health Reports *54*, 1880 (1939).

[47] D. D. WOODS and P. FIELDES, J. Soc. Chem. Ind. *59*, 133 (1940).

[48] O. DANN and E. F. MÖLLER, Ber. Dtsch. Chem. Ges. *82*, 76 (1949).

[49] R. L. MAYER, Science *98*, 203 (1943); J. Bact. *48*, 337 (1944); J. Bact. *48*, 93 (1944); Nature, *165*, 37 (1950).

[50] K. LANDSTEINER, *The Specificity of Serological Reactions* (Cambridge, 1945).

(a) *Tests with coupling products*

If the directly active, metabolic transformation product were a coupling product such as acetyl amino derivatives, one would expect that it would produce stronger skin reactions than the non-acetylated, free amines. Skin tests performed with acetyl or diacetyl derivatives of various aromatic amines and azo-dyes on humans or animals sensitive to the free amines have never produced stronger reactions, but rather regularly weaker reactions than the unsubstituted amines (MAYER[51]). From these results it is concluded that this prototype of metabolite does not constitute the direct active antigen and common immunologic property.

(b) *Skin tests with intermediate transformation products of azo-compounds*

Skin tests with hydrazobenzene, oxyazobenzene and 2-4-diaminoazobenzene on PPD-sensitive patients were negative; this has therefore led to the conclusion that no partially reduced or oxidized azo compound of the above-mentioned constitution acts as the common offender in a cross-sensitization in which PPD participates (MAYER[52]).

As shown in the preceding section, hydroxylamines and compounds of quinone structure are probable intermediates formed from various aromatic amines, both groups of substances being very similar from a toxicological standpoint: they are highly reactive and capable of rapidly transforming hemoglobin into methemoglobin. But the fact that these substances have the same pharmacological and toxicological activities does not permit any conclusion as to whether both are involved in this cross-sensitization. On the contrary, pharmacological activity depends upon mechanisms quite different from those involving immunological activity (DOERR[53]).

Table IV

Skin tests with various possible metabolites of aromatic amines on patients sensitive to aromatic amines (MAYER[52, 54]).

p-Phenylenediamine	+ + + +
Amino-azobenzene	+ + + +
Phenylhydroxylamine	0
Tolylhydroxylamine	0
Azoxybenzene	0
Quinone-diimine	+ + + + + +
Quinone-dichlorimine	+ + + + + + +
Bandrowski's base	+ + + +
Benzoquinone	+ +

In order to identify the chemical nature of the actual antigen and decide the question as to whether hydroxylamines, compounds of quinone structure, or both represent the common denominator in this cross-sensitization, the skin reactivity of individuals and animals sensitive to PPD and azo-dyes was tested with phenylhydroxylamines, tolylhydroxylamine, azoxybenzene, quinone diimine, quinone dichlorimine, Bandrowski's base and benzoquinone. The results obtained are shown in Table IV.

Positive reactions were elicited by quinone diimine, quinone dichlorimine, Bandrowski's base (an oxidation product of PPD inadequately defined chemically and long considered as being a non-irritant end-product of the oxidation of PPD) and benzoquinone. Negative reactions were obtained with the two hydroxylamine derivatives and azoxybenzene. The negative results obtained with phenyl- and tolyl-hydroxylamine strongly suggested that an oxidation of aromatic amines to hydroxylamines or azoxy derivatives was not associated with the antigenic properties of the substances under discussion, although it is supposed to play a major role in the case of methemoglobin formation.

The skin-reactions produced by quinone diimine and quinone dichlorimine, on the contrary, were extremely severe and many times stronger and more persistent than those observed after tests with identical amounts of PPD and aminoazobenzene. Thus the conclusion seems justified that the directly active substances responsible for the allergic cross-sensitization between PPD and azo-dyes are compounds of quinone structure, particularly quinone diimine.

The *inclusion of sulfonamides and PABA* in a cross-sensitization in which compounds of quinone structure are likely to represent the actual antigens indicates the direction of further investigations on the metabolic fate of these two substances. It is very probable that certain oxidation products such as compounds of quinone structure are formed when sulfonamides as well as PABA are ingested either after previous formation of hydroxylamines or by direct oxidation.

The fact that these postulated intermediates have not been found thus far constitutes no proof that they do not exist. Since immune reactions can be produced by extremely small and chemically undetectable amounts of antigen, the amounts of antigenically active oxidation products formed in the body may be too small to elicit recognizable *toxic* symptoms, although they are large enough to produce sensitization and allergic manifestations.

It must be realized that the present study concerns itself only with the role of compounds of quinone structure in allergy. These experiments do not constitute any direct evidence that compounds with a quinone structure are responsible, for instance, for the chemotherapeutic activity of sulfonamides or PABA.

In Fig. 2 an attempt is made to correlate the metabolic chemical transformation of the various aromatic

[51] R. L. MAYER, Arch. Dermatol. Syph. *163*, 223 (1931).
[52] R. L. MAYER, Arch. Dermatol. Syph. *163*, 223 (1931).
[53] R. DOERR, Arch. Dermat. Syph. *151*, 7 (1929).
[54] R. L. MAYER, Klin. Wschr. 1, 2. Hälfte 1958 (1928); Arch. Dermatol. Syph. *158*, 266 (1939).

amines, which may explain their antigenic nature and their participation in a cross-sensitization.

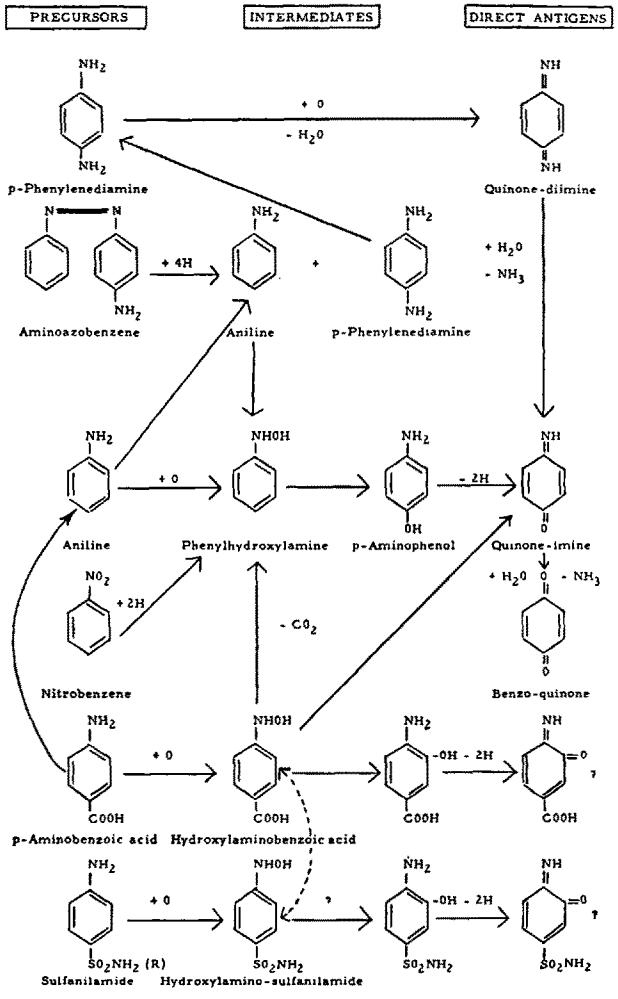


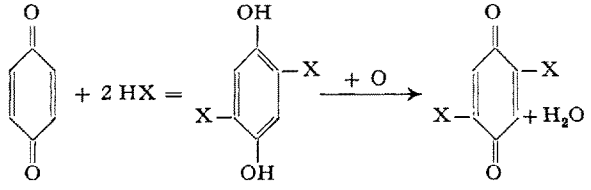
Fig. 2.

8. *Mechanism of action*

III. Transformation of the metabolically formed substances into complete antigens

Compounds of low molecular weight are incomplete antigens or "haptens"; they are unable to sensitize unless attached to a carrier of high molecular weight. Only then do they acquire full antigenicity (KLOPSTOCK and SELTER[55], LANDSTEINER[56], HEIDELBERGER and KENDALL[57]). The metabolic transformation products of PPD, azo-dyes and other substances into compounds with a quinone structure are still incomplete antigens or "haptens", as are the amines, azo-dyes, or nitro compounds from which they are derived. The question thus arises as to how they become attached to a carrier.

Quinone compounds possess great chemical avidity for combining with a variety of substances (POSNER[58], FISCHER and SCHRADER[59], MARTYNOFF and TSATSAS[60]), such as aniline, amines, sulfhydryl compounds, amino acids, alcohols, and many others, according to the following reactions:–



In this process the quinone is converted to hydroquinone but can be subsequently re-oxidized to a substituted quinone. The polymerization of these substituted quinones finally leads to compounds of high molecular weight.

Proteins are among the various body constituents most readily attached to quinones (SUIDA *et al.*[61]). Quinone imines and quinone diimines behave in this respect like benzoquinones and their affinity for various proteins such as animal cells, serum, etc. is especially apparent since the polymerization products resulting from this combination are intensely colored. Very conspicuous is the formation of almost black combination products of quinone diimine with SH-containing proteins, particularly keratin, a reaction which has become of great practical importance in the wide use of PPD and other amines and of their oxidation products, as fur and hair dyes.

The combination of quinones, quinone imines, or quinone diimines with proteins is very rapid. It is this avidity for protein carriers which, in my opinion, not only enables this group of substances to act as sensitizers, but also explains the great power to sensitize.

9. *The role of compounds of quinone structure in the cytologic changes following absorption of aromatic amines*

In addition to methemoglobin formation and allergic sensitization, certain aromatic amines, azo-dyes and quinones possess another apparently specific biological property: they can produce atypical epithelial proliferations and in some instances malignant growth. The conditions under which these cytological changes occur are indicative of similar metabolic oxidations and transformations to compounds of quinone structure, as disclosed in the case of allergy (cancer of the

[55] A. KLOPSTOCK and G. E. SELTER, Z. Immunitätsforsch. exper. Therap. *55*, 118 (1928).

[56] K. LANDSTEINER, Z. Immunitätsforsch. exper. Therap. *62*, 128 (1929).

[57] M. HEIDELBERGER and F. E. KENDALL, Proc. Soc. Exp. Biol. and Med. *26*, 482 (1929).

[58] T. POSNER, Liebigs Ann. Ch. *336*, 84 (1904).

[59] E. FISCHER and H. SCHRADER, Ber. Dtsch. Chem. Ges. *43*, 525 (1910).

[60] M. MARTYNOFF and G. TSATSAS, Bull. Soc. Ch., France, 1947, p. 52.

[61] H. SUIDA and W. SUIDA, Liebigs Ann. Ch. *416*, 113 (1918). Hoppe-Seylers physiol. Chemie *85*, 308 (1913).

bladder, oviduct, hepatoma, sarcoma, etc.) (FISCHER[62], REHN[63], HUEPER[64], YAMAGIWA and OHNO[65], SASAKI and YOSHIDA[66], KINOSITA[67], RHOADS[68], KIRBY and PEACOCK[69], HAEREM[70]).

In a previous study (MAYER[71]) the metabolic changes of various amines and azo-dyes which accompany or precede this cytologic action have been studied and it was found that the same conditions that are decisive for development of their antigenic nature also determine their action upon epithelial cells. Atypical growth induced by aromatic amines is dependent upon the formation of compounds of quinone structure from the amines or azo-dyes after destruction of the azo linkage and upon the ability of the quinone compound thus formed to combine with cell components (MAYER[71]) Indeed, (1) aromatic amines capable of producing atypical epithelial proliferations are antigenic; conversely those that are incapable of producing cell proliferation cannot induce allergic symptoms; (2) 2,5-diaminotoluene, which is a strong sensitizer, also produced considerable atypical epithelial reactions whereas 2,5-diamino-1,4-xylene, immunologically indifferent in cases of PPD sensitization, was incapable of producing precancerous growth (see Table V).

Table V
Substances tested for allergenic and cell proliferative properties (MAYER[71])

	Allergy	Epithelial proliferations
p-Phenylenediamine	+ + +	+ + +
m-Phenylenediamine	0	0
2–5-Diaminotoluene	+ + +	+ + +
2–5-Diamino-1–4-xylol	0	(0)
Nigrosine	0	0
Benzolazodiphenylamine	0	(0)
Diacetyl aminoazobenzene	+ + +	+ + +
Aminoazobenzol-2-naphthylamine	+ + +	+ + +

In spite of this basic similarity in the metabolic reactions leading to the two types of reactions, there is apparently an important difference between the immunological and cytological activities of the compounds of quinone structure: the "carriers" with which they combine seem to be different in the two instances. It is known from LANDSTEINER's experiments that in an allergic reaction the chemical nature of the carrier is irrelevant; identical sensitizations in *in vitro* immune reactions can be produced by haptens fixed to very different foreign or body proteins. But in the case of cell proliferation the chemical nature and physiological role of the "carrier" to which the quinone compound becomes fixed seems of greatest importance, e.g. fixation of the active principle to certain constituents of the cell nucleus seems to be necessary for the development of the reaction. There are many indications that atypical cell growth is caused by disturbances in the nuclear organization of the cell.

10. *Reaction between compounds of quinone structure and nuclear material*

It has been known for a long time that aromatic diamines, especially PPD and its di- and tetramethyl derivatives, specifically stain cell nuclei (WURSTER[72], UNNA[73]). The color develops slowly and is apparently dependent upon an oxidation of the staining material. This reaction is explained by the fact that quinone rapidly combines with purified desoxyribonucleoproteid, or with organized nucleoproteids such as crystalline tobacco mosaic virus[74].

Like the viruses, chromosomes contain pure nucleoproteids of different constitution, especially desoxyribonucleoproteid. Therefore chromosomes of the salivary glands of *Drosophila robusta* are progressively and specifically stained by aqueous solutions of quinone diimine. During this reaction the characteristic chromosome bands take on a purplish-brown to almost black color, whereas the protoplasm remains uncolored (MAYER[75]), as seen in Fig. 3.

Since disturbances in the structure and behavior of chromosomes are considered to be the principal causes of atypical cell proliferation and cancer, this affinity of quinone diimine for chromosomes may therefore constitute an important factor in the action of certain aromatic amines and azo-dyes on atypical cell growth.

The question obviously arises as to whether a formation of compounds of quinone structure and their fixation to nuclear material as a probable cause of atypical epithelial proliferation and subsequent malignancy are restricted to the metabolites of aromatic amines and azo-dyes, or whether a similar process can occur with other cancerogenic substances such as phenols, naphthyl derivatives, hydrocarbons, methylcholanthrene and steroid hormones. This is quite pos-

[62] B. FISCHER, Münch. med. Wschr. *53*, 2041 (1906); Frankfurt, Z. Pathol. *27*, 98 (1922).
[63] REHN, Arch. Klin. Chir. *50*, 588 (1895).
[64] W. HUEPER, J. Indust. Hyg. and Toxicol. *20*, 46 (1938).
[65] YAMAGIWA and OHNO, Jap. Z. Krebsf. *12*, 00 (1918).
[66] F. SASAKI and T. YOSHIDA, Virchows Arch. path. Anat. *295*, 175 (1935).
[67] R. KINOSITA, Trans. Japan. Path. Soc. *27*, 665 (1937).
[68] C. P. RHOADS, Bull. New York Acad. Med. *18*, 53 (1942).
[69] A. H. M. KIRBY and P. P. PEACOCK, J. Path. and Bact. *59*, 1 (1947).
[70] A. T. HAEREM, Proc. Soc. Exp. Biol. and Med. *45*, 536 (1940); *68*, 330 (1948).
[71] R. L. MAYER, Arch. Gewerbepath. Gewerbehyg. *1*, 436 (1930).
[72] C. WURSTER, Ber. Dtsch. Chem. Ges. *19*, 3195, 1886; *20*, 256 (1887).
[73] P. G. UNNA, Mh. prakt. Dermat. *6*, 243 (1887).
[74] We are indebted to Drs. STANLEY and MALKIEL, Rockefeller Institute, Princeton, for supplying the tobacco mosaic virus used in this study.
[75] R. L. MAYER, Proc. Soc. Exp. Biol. and Med. *68*, 664 (1948).

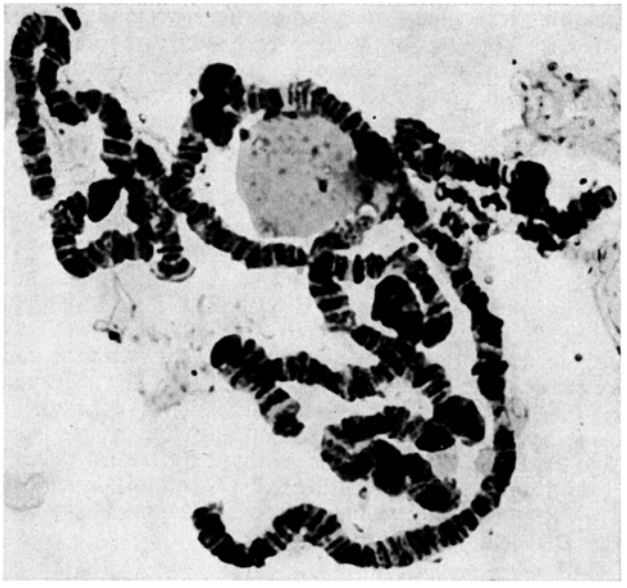


Fig. – 3. Chromosomes of the salivary gland of *Drosophila robusta*, stained with quinone diimine (MAYER[76]).

sible, for it is known that many of these cancerogens are metabolically oxidized to dihydroxy derivatives and quinones and their metabolic behavior is in many respects similar to that of aromatic amines (BOYLAND and co-workers[77], CASON and FIESER[78], DOBRINER *et al.*[79], WEIGERT[80], BERENBLUM *et al.*[81], BOYLAND and LEVI[82]). Benzoquinone, for instance, has a specific affinity to nuclear material and its cancerogenic properties have been described by TAKIZAWA[83].

11. *The allergic and cytologic reactions as a biological test for metabolic intermediates*

It is obvious that the high specificity of the immunological and cytological reactions can serve as a biochemical tool which permits more precise chemical characterization of unknown metabolites than do other biological, especially toxicological reactions such as development of methemoglobinemia. The inclusion of sulfonamides and derivatives of PABA in an immunological system based upon the presence of intermediate oxidation products of quinone structure is the first example of its practical usefulness. Indeed, the positive allergic reactions produced by sulfonamides and PABA in individuals specifically sensitive to aromatic amines prove that chemical similarities must exist between their respective metabolites. In this way the allergic reaction may serve as a basis for the detection of biochemical relationships in the same capacity as do the genetical particularities of Neurospora (BEADLE and TATUM[84, 85]) or the biochemical reactions of bacterial enzymes, as shown by STANIER[86]. The question as to the metabolic formation of a hydroxylamino sulfonamide or similar compound with subsequent oxidation into a quinone imino sulfonamide or its direct formation requires serious consideration. Such a transformation, previously discussed in connection with toxicologic and chemotherapeutic phenomena, is now again postulated from the immunological behavior of sulfonamides. Intimately associated with the question of sulfonamide metabolites is that of the still unknown constitution of biologically active transformation products of PABA.

12. *Conclusion*

The "cross-sensitization to compounds of quinone structure" is an allergy produced by a large group of various aromatic compounds: aromatic amines and nitro derivatives, certain azo-dyes, sulfonamides, p-aminobenzoic acid and its derivatives, and in some instances to certain polyphenols.

Experimental sensitizations of animals and testing of allergic individuals strongly suggest that the common property of the various chemical substances involved in this special group of allergies is represented by the metabolic formation of oxidation or reduction products, most likely compounds of quinone structure. It is suggested that these metabolites, rather than the original amines, nitro compounds, azo-dyes, sulfonamides or PABA derivatives represent the direct, active antigens. The high affinity of these metabolites for proteins, together with certain still unknown specific properties of quinone compounds and related substances, explain the particularly high sensitizing power of the antigens involved in this allergy.

It is likely that several members of this same group of substances are able to produce atypical epithelial proliferations and malignant growth through a similar mechanism; the metabolic transformation products and not the original, unaltered amines and the strong affinity of the quinoid metabolites for nuclear material, especially chromosomes, appear to be the cause of the cytological changes.

Zusammenfassung

Gewisse aromatische Amine, Diamine und Nitroverbindungen, Aminophenole und amidierte Azofarbstoffe sind starke Allergene und verursachen häufig allergische Überempfindlichkeitsreaktionen, wie Der-

[76] R. L. MAYER, Arch. Gewerbepath. Gewerbehyg. *1*, 436 (1930).
[77] E. BOYLAND, A. A. LEVI, E. H. MAWSON, and E. ROE, Biochem. J. *35*, 184 (1941).
[78] J. CASON and L. F. FIESER, J. Amer. Chem. Soc. *62*, 2681 (1946).
[79] K. DOBRINER, C. P. RHOADS, and G. I. LAVIN, Proc. Soc. Exp. Biol. and Med. *41*, 67 (1939).
[80] F. WEIGERT, Nature *155*, 479 (1945).
[81] I. BERENBLUM and R. SCHOENTAL, Cancer Res. *3*, 145, 688 (1943).
[82] E. BOYLAND and A. A. LEVI, Biochem. J. *29*, 2677 (1935); *30*, 728, 1225 (1936).
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[84] G. W. BEADLE and E. L. TATUM, Amer. J. Botany *32*, 678 (1945).
[85] E. L. TATUM, Cold Spring Harbor Symposia Quant. Biol. *11*, 278 (1946).
[86] R. STANIER, 49th Meeting Soc. Amer. Bact., Cincinnati, 1949.

matitis, Konjunktivitis, Urtikaria, Asthma. Die Allergien, welche durch diese verschiedenen Substanzen hervorgerufen werden, sind von dem Autor als «Gruppenüberempfindlichkeit gegen Körper von Chinonstruktur» zusammengefaßt, weil die eigentlichen und direkten Antigene nicht die Amine oder Nitrokörper als solche, sondern Chinonkörper sind, entstanden durch die Oxydation der Amine im intermediären Stoffwechsel.

Es hat sich gezeigt, daß in vielen Fällen von Überempfindlichkeit gegen Paraphenylendiamine auch Sulfonamide und Paraaminobenzoesäure als spezifische Antigene wirken und Hautüberempfindlichkeitserscheinungen hervorrufen. Es muß daher angenommen werden, daß auch Sulfonamide und Paraaminobenzoesäure im Stoffwechsel in Chinonkörper umgewandelt werden können. In der Tat konnte die Bildung derartiger metabolischer Oxydationsprodukte unter verschiedenen Versuchsbedingungen wahrscheinlich gemacht werden.

Alle hier genannten chemischen Substanzen sind selbst nach oxydativer Umwandlung in Chinonkörper unvollständige Antigene, sogenannte Haptene. Nach Verbindung mit hochmolekularen «Schleppersubstanzen» werden Haptene in Vollantigene umgewandelt. Die Chinonkörper haben eine sehr große Affinität zu verschiedenen hochmolekularen Bestandteilen des tierischen Organismus, wie z. B. Polypeptide, Proteine, Lipoide, usw., die bekanntlich als Schlepper wirken können. Durch chemische Verbindung der Chinonkörper mit diesen Körperbestandteilen und nachfolgender Polymerisation der Verbindungsprodukte, z. B. von Chinon/Eiweiß-Verbindungen, entstehen Vollantigene.

Es ist bekannt, daß eine große Anzahl der gleichen Amine und Azofarbstoffe Krebserzeuger sind (Anilinkrebs, Buttergelbkrebs), und fast alle aromatischen Amine können atypische epitheliale Wucherungen hervorrufen. Wie im Falle der sensibilisierenden Wirkung ist die Erzeugung atypischer epithelialer Wucherungen von der metabolischen Umwandlung der Amine und Azo-Farbstoffe in Chinonkörper abhängig. In diesem Falle jedoch scheint die Affinität der Chinonkörper zu den Nukleoproteiden der Chromosomen von ausschlaggebender Bedeutung zu sein.

Die Paläoneurologie am Beginn einer neuen Phase

Von TILLY EDINGER, Cambridge, Mass., USA.[1]

«Fossile Gehirne»

Wie man lange Zeit nach bestem Wissen sagen konnte «L'homme fossile n'existe pas», so muß man heute gestehen: Fossile Gehirne gibt es nicht. Mumifiziert und geschrumpft in trockenem Klima oder gegerbt und verquollen im Moor hat sich Gehirnsubstanz jahrtausendelang erhalten. Fossilisation ist demnach möglich. Aber alles, was gelegentlich als versteinertes Gehirn beschrieben worden ist, war entweder *lusus naturae* oder die gehirnförmige Steinausfüllung einer fossilen Schädelhöhle, deren lebendiger Inhalt schon verwest war, als der Schlamm oder Sand eindrang, der später zu Stein erhärtete. Solche Steinkerne oder auch künstliche Schädelhöhlenausgüsse meinen wir, wenn wir leichthin von fossilen Gehirnen sprechen. In fossilen Schädeln erhielt sich zwar keine so oder so veränderte Gehirnsubstanz, unverändert aber die Form der Vorzeitgehirne – dadurch daß es ja einst das lebende Gehirn war, das der Innenseite der Schädelkapsel ihre Form gegeben hat.

In der Klasse Mammalia (von der insbesondere hier die Rede sein wird) haben in den meisten Ordnungen all die Teile des Gehirns, die von außen sichtbar sind, durch die Meningen hindurch jedes Detail ihrer Oberfläche dem Schädel eingeprägt. Durch die vergleichende Anatomie der rezenten Wirbeltiere wissen wir, in welch hohem Maße am Gehirn die Form die mikroskopische Struktur, das so sehr verschiedene Größenverhältnis der Teile die jeweilige Bedeutung ihrer respektiven Funktion widerspiegelt. Es ist dadurch völlig klar, daß auch an Hirnschädelausgüssen beispielsweise nicht nur relativ große Riechbulbi scharfem Geruchssinn des ausgestorbenen Tiers entsprechen, sondern natürlich auch, wie bei einem wirklichen Gehirn, ein ausgedehntes und gefurchtes Neopallium mehr Möglichkeit zu geistigen Funktionen – nämlich größere zytoarchitektonische Differenzierung als bei einem kleinen glatten Neopallium – bedeutet. Die Paläoneurologie hat sich um Schlüsse auf Details der ja nichterhaltenen inneren Struktur bisher kaum bemüht; aber zu solchen ist sie nunmehr auch berechtigt.

Bisher...

Leser der «Experientia» kennen aus der Paläoneurologie möglicherweise nur KAPPERS' Beitrag zu dem der Stammesgeschichte des *Menschen* gewidmeten Heft (Vol. II, Fasc. 8, 1946). Manche mögen sich gefragt haben, ob solcherlei Forschung denn irgendwelchen Wert hat – weshalb sich sogar dieser größte Hirnanatom unserer Zeit mit den Durchmesserindices und der Anordnung der Furchen am Stirnlappen fossiler Hominiden be-

[1] Museum of Comparative Zoology at Harvard College.– These investigations are beeing aided by a grant from the Milton Fund of Harvard University.