NEW PENETRATING VEHICLES AND SOLVENTS[1]

THIS is a preliminary report on the development of new penetrating vehicles and solvents. Our experiments demonstrate that these new vehicles and solvents are capable of carrying a great variety of substances into and through grossly intact living tissues at a greater rate of speed than heretofore possible; and also of achieving significant penetration by some substances which could hitherto not be made to penetrate intact human skin to a demonstrable degree.

These experiments, carried on during several years, consisted at first in studying the rate of penetration of certain substances (*e.g.*, sulfonamides, bismuth, mercury, iron) into "colloid models." For these models we used gelatine (simulating the natural protein-gels) and fats, such as wool fat, petroleum jelly and aquaphore (Duke) (simulating the natural lipids).

Specific reagents were added to the models to serve as "indicators"; their characteristic color-changes demonstrated the rate of penetration, the distribution and the chemical reactivity of the various substances embodied in the vehicles under investigation. For example, dimethylaminobenzaldehyde was incorporated in the gelatine models to indicate the penetration, if any, of sulfonamides; potassium iodide or ammonium sulfide to demonstrate penetration of metals such as bismuth or mercury.

A long series of these model experiments revealed that, of all the vehicles and solvents tested, the most massive as well as the speediest penetration was achieved with a combination of: (1) propylene glycol, (2) antipyrine, (3) certain sulfonated wetting agents and (4) xylene or mesitylene. Vehicles composed of such combinations of ingredients were regularly observed not only to effect the most rapid penetration of the incorporated substance, but also to preserve its chemical reactivity, as manifested by the behavior in both the fat and the protein factors of the models.

It was found that the substitution of even closely related chemicals—such as toluene for the xylene or mesitylene—produced a vehicle which did not promote any visible penetration into or reactivity in the fatty medium. Moreover, the presence of certain wetting agents was found to be indispensable to achieve the desired effects in both the fatty and the protein media.

Under the conditions of our experiments, the following wetting agents were found to be useful: (1) Dihexylester of sodium sulfosuccinic acid (Aerosol MA) and (2) dibutylester of sodium sulfosuccinic acid (Aerosol 1 B). The vehicles were also very efficient when parasodium xylene sulfonate was used in place of the combination of xylene and the sulfonated wetting agents just mentioned.

The role of the antipyrine in our combination was not only that of a surface-active agent, but also of an exceptionally good means of increasing solvent action. For example, the hitherto poorly soluble sulfonamides (bases) can be brought into solution in almost any desired concentration through the action of the antipyrine. Moreover, the antipyrine is an indispensable ingredient because of its capacity of transforming the heterogeneous phases into one homogeneous solution. In our early *in vitro* experiments, we had observed that, in the presence of aerosols in the amount used, it was impossible to make a homogeneous solution of propylene glycol and the required quantity of xylene; this difficulty was overcome by the addition of antipyrine. Furthermore, we succeeded in making xylene water-soluble by adding one of the above-mentioned aerosols plus antipyrine.

Three principal combinations, subject to modification, were eventually selected as optimal for studies in penetration through human and animal skin. For the sake of brevity, we have coined the generic term of "Penetrasol" to designate these new vehicles and solvents.

Penetrasol A. Aerosol MA one part by weight, *e.g.*, 20 grams
Xylene one part by volume, *e.g.*, 20 cc
These two components are warmed under reflux. A glassy gel results. This gel is taken up with
Antipyrine one part by weight, *e.g.*, 20 grams
in Propylene glycol, four parts by volume, *e.g.*, 80 cc

Penetrasol B. Same as Penetrasol A, except for the substitution of Aerosol 1 B for Aerosol MA.
(Mesitylene can be substituted for Xylene in Vehicles A and B)

Penetrasol C. Sodium paraxylene sulfonate, one part by weight, *e.g.*, 20 grams
Antipyrine, one part by weight, *e.g.*, 20 grams
in Propylene glycol, 5 parts by volume, *e.g.*, 100 cc

The above described Penetrasols have been employed by us in certain fairly extensive experiments on penetration of living human and animal skins. The results were roughly such as to be expected from our *in vitro* studies.

Thus, the use of Penetrasols with the usual "protein allergens," applied to grossly intact human skin, produced wheals in specifically hypersensitive human subjects. Gentle rubbing for 30 seconds on the unbroken,

[1] The reported experiments were aided by grants from the Dr. Simon Baruch Foundation and from the Wallace Laboratories of New Brunswick, N. J.

normal skin site with a drop of the appropriate Penetrasols to which the powdered specific allergen had been added, produced whealing in over 99.5 per cent. of the tests with those allergens which regularly elicited wheal reactions when applied to the same patient by the ordinary scratch-test method. Every allergen was tested with Penetrasol A, B and C in every individual examination. In most instances, all three vehicles were found to be effective. An interesting finding was that Penetrasols A and B (more lipid-soluble) were superior in carrying pollen, silk, kapok and foods through the human skin; while the more hydrophile Penetrasol C was more effective in dealing with horny materials (*e.g.*, danders), as well as with house dust, cotton and woods.

The penetration of metallic salts and of sulfonamides into and through grossly intact human and animal skin was demonstrated by means of histologic studies and histochemical "indicators." In some instances, new methods were developed to facilitate demonstration of the route and the extent of the penetration. For example, a newly developed histochemical demonstration of sulfonamides employing a solution of 1 per cent. p.-dimethylaminobenzaldehyde in absolute ethylalcohol containing 5 per cent. concentrated hydrochloric acid, will be described in a subsequent, more detailed report.

To date, our histochemical studies have demonstrated the penetration of the intact human skin by: (1) iron (ferric chloride, and ferric ammon. citrate); (2) bismuth (Sobisminol); (3) sulfanilamide. Neither equally concentrated solutions in propylene glycol nor aqueous solutions nor dispersions in lanolin produced such demonstrable penetration into or through intact human or animal skin.

Still another method for demonstrating the penetration was employed in studying the absorption of various sulfonamides (sulfanilamide, sulfathiazole). Inunctions of areas of intact skin of 11 human volunteers were carried out with not more than 1 gram of the sulfonamide in a vehicle of type of Penetrasol A, and repeated in about two hours. Penetration was proved to have taken place by demonstration of the free drug, in the blood and urine. In general, the drugs were demonstrated by a modification of Werner's method. The maxima were reached within one half to three hours after the applications.

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ON HUMAN ANTI-RH SERA AND THEIR IMPORTANCE IN RACIAL STUDIES[1]

SOON after the importance of the Rh factor in erythroblastosis fetalis and in transfusion accidents was established,[2, 3] it was observed that various human anti-Rh sera do not exhibit identical specificities.[3-7] Until recently the three main varieties were identified by their characteristic percentages of positive reactions obtained in the white race, *i.e.*, 87 per cent., 85 per cent. and 73 per cent., respectively.[3-8] In agreement with Landsteiner and Wiener these antibodies may now be termed anti-$Rh_{1.2}$, anti-Rh_1 and anti-Rh_2, respectively. This terminology is based on the observation of Levine[8] that the anti-Rh serum which gives 87 per cent. reactions, in contrast to the others, contains more than one antibody. The anti-Rh_1 serum may be considered as standard since it has a specificity practically identical with that of the experimental serum of Landsteiner and Wiener.‡

From the point of view of diagnosis of erythroblastosis fetalis and the prevention of intra-group transfusion accidents, either one of the two varieties

TABLE 1

TESTS WITH ANTI-Rh_1 SERUM

Race	Number tested	Percentages +	Percentages −
White[a]	334	85	15
Colored[b]	264	95.5	4.5
Colored[c]	113	92	8
American Indians[d]	120	92.2	0.8
Chinese[e]	150	99.3	0.7

[a] Levine, Burnham, Katzin and Vogel.[3]
[b] Levine in this study.
[c] Landsteiner and Wiener.[6]
[d] Landsteiner, Wiener and Matson.[9]
[e] Levine and Wong.[10]

[1] Aided by grants from the Blood Transfusion Association in New York City and the National Committee on Maternal Health.

[2] P. Levine, E. M. Katzin and L. Burnham, *Jour. Am. Med. Asn.*, 116: 825, 1941.

[3] P. Levine, L. Burnham, E. M. Katzin and P. Vogel, *Am. Jour. Obst. and Gyn.*, 42: 925, 1941.

[4] A. S. Wiener, *Arch. Path.*, 32: 227, 1941.

[5] P. Levine, E. M. Katzin, P. Vogel and L. Burnham, Chapter XXXI in "Blood Substitutes and Blood Transfusions." C. C Thomas, Springfield, Ill.

[6] K. Landsteiner and A. S. Wiener, *Jour. Exp. Med.*, 74: 309, 1941.

[7] I. Davidsohn and B. Toharsky, *Am. Jour. Clin. Path.*, 12: 434, 1942.

[8] P. Levine, *New York State Jour. of Med.*, 42: 1928, 1942.

[9] K. Landsteiner, A. S. Wiener and G. A. Matson, *Jour. Exp. Med.*, 76: 73, 1942.

[10] P. Levine and H. Wong, *Am. J. Obst. and Gyn.* In press.

‡ Anti-Rh_2 sera were observed independently first by Wiener (*Arch. Path.*, 32: 227, 1941) and shortly thereafter by Levine.[3]