Sadana, J. C. & Ahmad, B. (1948). *J. sci. industr. Res.* **7B**, 96.
Sadana, J. C. & Ahmad, B. (1949*a*). *Indian J. med. Res.* **37**, 193.
Sadana, J. C. & Ahmad, B. (1949*b*). *J. sci. industr. Res.* **8B**, 35.
Sherman, W. C. (1941). *Proc. Soc. exp. Biol., N.Y.*, **47**, 199.
Sinclair, H. M. (1953). *Proc. Nutr. Soc.* **12**, 69.
Squibb, R. L., Cannon, C. Y. & Allen, R. S. (1948). *J. Dairy Sci.* **31**, 421.
Steinegger, P., Streiff, K. & Zeller, P. (1957). *Mitt. Lebensm. Hyg., Bern*, **48**, no. 6.
Steven, D. M. (1948). *J. exp. Biol.* **25**, 369.
Strauss, W. (1942). *Helv. chim. acta*, **25**, 705.
Subrahmanyan, V., Narayanarao, M., Ramarao, G. & Swaminathan, M. (1955). *Brit. J. Nutr.* **9**, 350.
Thompson, S. Y. (1963). In *Carotine und Carotenoide*, p. 263. [K. Lang, editor.] (*Wissenschaftliche Veröffentlichungen der Deutschen Gesellschaft für Ernährung*. Vol. 9.) Darmstadt: Dr Dietrich Steinkopff Verlag.
Thompson, S. Y. (1964). *Exp. Eye Res.* **3**, 392.
Thompson, S. Y., Henry, K. M. & Kon, S. K. (1964). *J. Dairy Res.* **31**, 1.
Thompson, S. Y. & Kon, S. K. (1950). *Congr. int. Sucr. Distill.* VIII. *Brussels,* **2**, 55.
Tomarelli, R. M., Charney, J. & Bernhart, F. W. (1946). *Proc. Soc. exp. Biol., N.Y.*, **63**, 108.
United States Department of Agriculture (1957). *Household Food Consumption Survey* 1955. Reports no. 1 and no. 6. Washington: US Government Printing Office.
von Euler, H. & Klussman, E. (1932). *Hoppe-Seyl. Z.* **213**, 12.
Wald, G. & Zussman, H. (1938). *J. biol. Chem.* **122**, 449.
Winterstein, A., Studer, A. & Rüegg, A. (1960). *Chem. Ber.* **93**, 2951.
World Health Organization (1950). *Tech. Rep. Ser. Wld Hlth Org.* no. 3.
Zechmeister, L. (1949). *Vitam. & Horm.* **7**, 57.
Zechmeister, L., Le Rosen, A. L., Schroeder, W. A., Polgar, A. & Pauling, L. (1943). *J. Amer. chem. Soc.* **65**, 1940.
Zechmeister, L. & Petracek, F. J. (1956). *Arch. Biochem. Biophys.* **61**, 243.
Zechmeister, L. & Polgar, A. (1941). *J. biol. Chem.* **139**, 193.

Occurrence, absorption and distribution of vitamin A

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Vitamin A itself is found in the animal kingdom together with carotenoid pigments, but plants are considered to contain carotenoid pigments but no preformed vitamin A. A possible exception is the small amount of the aldehyde form of vitamin A found by Winterstein & Hegedüs (1960) in spinach and other plants; the concentrations present, of the order of $0 \cdot 1$ μg/g fresh weight, are not of nutritional importance, and the aldehyde is possibly there as a minor metabolite of the carotenoid pigments.

With vitamin A the occurrence and distribution are therefore closely related. By occurrence is meant here the presence in foodstuffs, particularly those of man, whereas distribution refers to the presence of vitamin A in different organs, tissues, cells and intracellular particles of man and other animals after its ingestion and absorption.

Occurrence

Forms of vitamin A. Some of the forms of vitamin A that occur naturally are shown in Fig. 1. The basic compound, retinol or vitamin A_1 alcohol, contains twenty carbon atoms arranged in a β-ionone ring with a side-chain of two isoprene units and a primary alcohol group. Five conjugated double bonds are present. A similar compound, dehydroretinol or vitamin A_2 alcohol, has two hydrogen atoms less in the
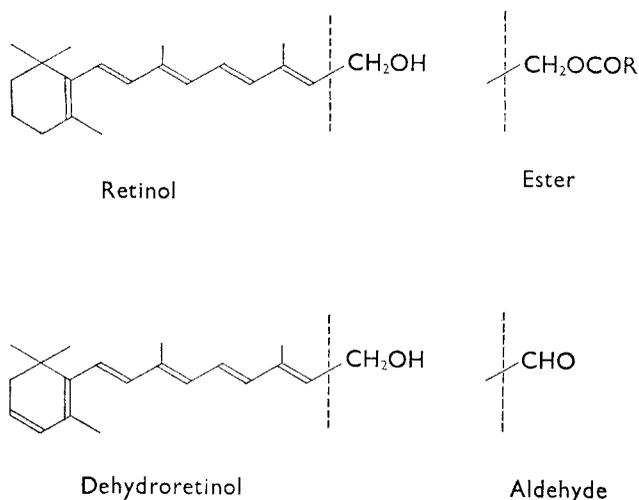
Fig. 1. Structural formulas of retinol, dehydroretinol and their ester and aldehyde derivatives.

ring and the extra double bond thus formed is conjugated with the other five. Aldehyde derivatives of these two forms are found and they are now given the names of retinal and dehydroretinal. The most commonly occurring forms, however, are the esters, formed by the combination of retinol or dehydroretinol with fatty acids.

In addition, *cis-trans* isomerism is possible about three of the double bonds, and six different geometrical isomers of retinol, of retinal and of retinyl esters are known, and presumably the same number exists of isomers of dehydroretinol, dehydroretinal and dehydroretinyl esters. Various other derivatives of retinol are known, for instance retinoic acid and anhydroretinol, but they occur naturally only in very small quantities, if at all.

Occurrence of these forms of vitamin A. From the viewpoint of the nutrition worker, this complex collection of different forms of vitamin A can be considerably reduced in number. The A_1 form of the vitamin represents 90 % or more of the intake of pre-formed vitamin A by man. The dehydro-, or A_2, form is usually predominant in freshwater fish and is present to the extent of about 25 % of the total vitamin A in some marine fish, but only when supplements of fish-liver oil are given is it likely to represent a significant part of the vitamin A consumed by man. Of the alcohol, ester and aldehyde forms of vitamin A, the ester form comprises more than 90 % of the vitamin A intake of man. This form is predominant in liver, kidney, milk and milk products, and fish, whereas the alcohol form, though present in small amounts in these foods, is the predominant form only in hen's eggs. The aldehyde form of vitamin A is of little nutritional importance for man, although as the predominant form of the vitamin in many fish eggs (Table 1) it may be important for fish embryos. The aldehyde is also present in birds' eggs (Table 1). Of the *cis-trans* isomers, again one particular form, the all-*trans*, predominates in the food of man. The 13-*cis*-,

Table 1. *Vitamin A in the eggs of fish and birds*

Fish	Retinal + dehydroretinal (μg/g dry weight)	Retinol + dehydroretinol and their esters (μg/g dry weight)
Cod	3·9	0·4
Herring	3·7	1·3
Plaice	5·3	0·5

Bird	Weight of egg (g)	Retinal (μg/egg)	Retinyl esters (μg/egg)	Retinol (μg/egg)
Domestic hen	65	22	25	122
Common goldeneye duck	51	51	31	31
Western greylag goose	158	128	67	196

Sources: Plack & Kon (1961); Plack (1963); Plack, unpublished observations.

or neo-form, is next in order of importance and the other *cis*-isomers normally occur as only a very small proportion of the total vitamin A. An interesting point that may be mentioned in passing is that certain marine Crustacea, some of which form the food of baleen whales, have most of their vitamin A stored in their eyes and the major part is the 11-*cis* isomer (Fisher, Kon & Plack, 1957), the geometrical form which is involved in the visual cycle, although the amount present is many times in excess of that required for the visual function (Fisher & Kon, 1959).

Thus, of the intake of preformed vitamin A by man, the major part is in the form of all-*trans* retinyl ester, and there is some evidence that the ester most commonly found is the palmitate (Mahadevan & Ganguly, 1961).

Vitamin A requirements of man. The determination of the precise vitamin A requirements of man has proved to be difficult, and the liberal values adopted by the Committee on Nutrition of the British Medical Association (1950) are now usually accepted in this country. In terms of i.u. vitamin A (as opposed to carotene) per day they are: children (15 years and under) 1500; adolescents and adults 2500; pregnant women 3000; lactating mothers 4000. The corresponding values recommended in the USA, converted into i.u. of preformed vitamin A per day, are: children 1500–3000; adolescents and adults 3000; pregnant women 3600; lactating mothers 4800 ((USA) National Research Council, 1964). One international unit of vitamin A is equivalent to 0·3 μg of retinol. Mean values for the total vitamin A intakes (preformed plus provitamin A) of different social classes in Great Britain in 1962 were all over 4000 i.u. vitamin A per day (Ministry of Agriculture, Fisheries and Food: National Food Survey Committee, 1964) so that, in this country at least, there is no general lack of vitamin A in the diet, although some individuals may suffer from an inadequate intake.

Table 2 gives the vitamin A content of common foodstuffs, arranged in order of concentration of vitamin A. This order does not necessarily represent the importance of these foodstuffs as sources of vitamin A in the diet (Table 3) although the first two places, filled by liver and butter, are the same in the two tables. Table 3 includes vegetable foodstuffs containing carotenoid pigments, and it can be calculated that the

Table 2. *Vitamin A in foodstuffs (i.u./100 g)*

Liver	4000–45 000
Butter	3500
Margarine	3000
Cheese	1400
Kidney	1000
Eggs	1000
Salmon	300
Herring	150
Milk	100–150*

Source: McCance & Widdowson (1960).
*i.u./100 ml.

Table 3. *Percentage of the total vitamin A intake of the British population contributed by various foodstuffs*

Liver	21
Butter	17
Carrots	13
Milk	12
Margarine	9
Eggs	8
Cheese	4
Other vegetables, fruit and other foods	16

Source: Ministry of Agriculture, Fisheries and Food: National Food Survey Committee (1964).

carotenoids provide about 33% of the total vitamin A intake for the average person in Great Britain, compared with about 67% as preformed vitamin A. This table also bears out the importance of fortifying margarine with vitamin A. Butter and margarine together provide 26% of the total vitamin A intake, and a family using margarine as fat to the exclusion of butter would lose a major source of vitamin A if the margarine were not enriched.

Absorption

Orally administered vitamin A is absorbed from the small intestine. Popper & Volk (1944) examined frozen sections of the intestines of rats after a massive dose of vitamin A. The vitamin was detected in the lymphatics of the small intestine by its fluorescence, and maximum fluorescence, and hence absorption, was found in the lymphatics between the upper and middle third of the intestine. Thompson, Braude, Coates, Cowie, Ganguly & Kon (1950) found maximum absorption in the second quarter of the small intestine of rats. Earlier, Drummond, Bell & Palmer (1935) had demonstrated that vitamin A is carried from the intestine by the lymphatic system, and later workers have confirmed this finding. The vitamin is then carried by the blood to the liver, which is the chief storage organ.

Much work has been done in the last 15 years to elucidate the processes concerned with the absorption of vitamin A. In the section on occurrence it was shown that in its natural form vitamin A is present in foods chiefly as all-*trans* retinyl esters. Such esters are hydrolysed to the free alcohol (probably in the lumen of the intestine),

absorbed, and re-esterified in the intestinal wall (Gray, Morgareidge & Cawley, 1940; Thompson, Ganguly & Kon, 1949; Eden & Sellers, 1950; Mahadevan, Seshadri Sastry & Ganguly, 1963*a*,*b*). Proof of this sequence has partly come from the use of esters of retinol other than the palmitate, since it is the palmitate which is formed predominantly on re-esterification, and it is as the palmitate that vitamin A passes into the lymphatics and through the blood to the liver (Mahadevan & Ganguly, 1961).

Other changes also take place during the process of absorption, for instance isomerization. Murray, Stainer & Campbell (1959) found with rats that isomerization of all-*trans* and of 13-*cis* vitamin A to mixtures of these two isomers took place in the stomach and in the liver. Plack (1959) found that 11-*cis* vitamin A isomerized to the all-*trans* form to some extent in the intestinal contents of rats, in the intestinal walls and in the liver. These *cis*-isomers would seem to exert their biological activity as the all-*trans* form, since this is always the predominant form found in the liver, and their different biological activities for rats, 75 % for 13-*cis* and 23 % for 11-*cis* relative to all-*trans* vitamin A (Ames, Swanson & Harris, 1955), may be due to a combination of the efficiency of uptake of the isomer and the efficiency of its isomerization.

Another interesting phenomenon associated with absorption is the biological activity of dehydroretinol, which Shantz & Brinkman (1950) found to be 40 % relative to retinol by a growth test with rats. Since the A_2 form is not converted into the A_1 form, either the dehydroretinol is absorbed less well than retinol, or it is absorbed as well but is less effective biologically, or there is a combination of these two effects.

Several different factors affect the absorption of vitamin A, and one that has received some attention in the last few years is the amount of protein in the diet. Arroyave, Viteri, Béhar & Scrimshaw (1959) found that kwashiorkor in children interfered with the absorption of vitamin A but normal absorption took place after the children had received for some days a diet of acidified half-skimmed milk. Other workers, for instance Friend, Heard, Platt, Stewart & Turner (1961), found a significant correlation between the concentration of serum vitamin A and that of serum albumin, so that when the level of serum albumin was reduced in animals on a protein-deficient diet, the level of vitamin A in serum was also reduced. Deshmukh, Malathi & Ganguly (1964) claimed that, with diets containing 5, 10 or 20 % casein, the efficiency of absorption of vitamin A by rats after a single massive dose increased progressively with the increase in dietary protein. Their results certainly showed differences in the liver stores of vitamin A over periods of up to 6 h, but 24 h after dosing there was little difference in the liver stores of rats on the three diets. These authors did, however, show that the activities of certain enzymes, possibly concerned with vitamin A metabolism, were progressively decreased by lowering the protein content of the diet.

The carrier in which the vitamin is given affects its absorption from the intestine. In most foods, vitamin A is present in solution in lipids but in some it may be present as a protein complex. In lipid solution, vitamin A is susceptible to oxidation by stale or rancid fats and this susceptibility is reduced by the presence of antioxidants, in

particular vitamin E. Bile salts and lecithin help in dispersing the fat in the intestine and in this way aid the absorption of vitamin A. In experimental work an aqueous dispersion of vitamin A, prepared with the aid of a non-ionic surface-active agent, is frequently used, and absorption from such a dispersion is found to be better than from an oily solution (Lewis, Bodansky, Birmingham & Cohlan, 1947). Such dispersions have also been used clinically in cases of impaired intestinal absorption (Lewis *et al.* 1947; Kramer, Sobel & Gottfried, 1947).

Distribution

When a rat is given a vitamin A-deficient diet, its liver store of the vitamin is first depleted, whilst the concentration in blood is maintained. The blood level then rapidly falls to nought, but during this time the visual activity is normal. Finally, the amount of vitamin A present in the eye falls to nought and the animal becomes night-blind (Dowling & Wald, 1958).

When a depleted animal receives a small oral dose of vitamin A, the lymph carries the vitamin A from the intestines and it is distributed by the blood. The absorbed part of the dose is probably distributed in various tissues of the body and cannot be adequately traced by techniques now available. A dose of about 100 i.u. vitamin A is needed before any can be detected in the liver of a rat (Davies & Moore, 1948; Plack, unpublished observations). These findings suggest that either the concentrations in tissues needed for normal growth are very small, or that the vitamin is present in an active form which has not yet been detected.

Liver storage of vitamin A is often used as a measure of the efficiency of absorption from the food, or, in comparison with pure vitamin A, of the vitamin A activity of a substance. The above remarks show, however, that erroneous results can easily be obtained. Ames & Harris (1956), in their biological assay of vitamin A based on liver storage by rats, found that the proportion of the dose stored in the liver is constant at about 67 % over the dose range 500–10 000 i.u., but other workers have found that this proportion may be 0 % at doses below 100 i.u. and 10 % or less with very high doses of vitamin A (Davies & Moore, 1948).

Nevertheless, amongst the vertebrates, the liver is the chief storage organ for vitamin A, and the predominant form present there is retinyl palmitate (Mahadevan & Ganguly, 1961), although retinyl stearate and oleate are also present in substantial amounts (Futterman & Andrews, 1964).

There has been some controversy about the presence of isomers of vitamin A in the liver. Murray *et al.* (1959) and Plack (1959) found that, even after dosing rats with individual *cis*-isomers, the vitamin A in the liver after a few days was almost all in the all-*trans* form, whereas Brown, Blum & Stern (1959) found considerable amounts of *cis*-isomers in fish-liver oils and suggested that an equilibrium mixture of isomers was present.

In the blood, the ester form of vitamin A is carried to the liver from the lymph and hence from the intestines, and the alcohol form is transported from the liver to other tissues. Usually the alcohol form predominates but, after a large oral dose of vitamin A, it may be the ester form. The concentration of alcohol in blood is thought to be

regulated by some mechanism as yet unknown; the alcohol and the ester are carried on different proteins.

Of the other organs and tissues, the kidneys often contain measurable amounts of vitamin A, and traces have been reported in lungs, adrenals and intraperitoneal fat. The eyes also contain small quantities of vitamin A, and these small amounts, of course, play a very important part in vision. Some animals have large amounts of vitamin A in unusual places, for instance the halibut has a high concentration in its viscera, particularly in the pyloric caecums (Glover & Morton, 1948); the fulmar petrel has vitamin A and carotenoids in its stomach oil (Rosenheim & Webster, 1927) and the euphausiids have the vitamin concentrated in their eyes (Fisher *et al.* 1957).

The distribution of vitamin A within tissues has been followed by fluorescent microscopy, particularly by Popper and his coworkers. In the liver, vitamin A is associated with the Kupfer cells and is present mainly in the small droplets of lipid (Popper & Greenberg, 1941). Applying the techniques of cell disintegration and differential centrifugation to liver, Krinsky & Ganguly (1953) found that most of the esters of vitamin A were present in the upper 'creamy' layer of the supernatant liquid whereas retinol was present in the supernatant liquid and in the microsomal fraction.

Summary

The British population derives most of its vitamin A preformed from other animals. Some comes directly from the liver stores of the animals, some indirectly from the milk and milk products, and all is utilized quite efficiently, probably up to 50 % of the vitamin in the diet being deposited in the liver, on the basis of experiments with animals. Although man is capable of converting provitamins A into vitamin A, we in this country depend largely on other animals, particularly the cow, to do this conversion for us.

REFERENCES

Ames, S. R. & Harris, P. L. (1956). *Analyt. Chem.* **28**, 874.
Ames, S. R., Swanson, W. J. & Harris, P. L. (1955). *J. Amer. chem. Soc.* **77**, 4134.
Arroyave, G., Viteri, F., Béhar, M. & Scrimshaw, N. S. (1959). *Amer. J. clin. Nutr.* **7**, 185.
British Medical Association (1950). *Report of the Committee on Nutrition.* London: British Medical Association.
Brown, P. S., Blum, W. P. & Stern, M. H. (1959). *Nature, Lond.,* **184**, 1377.
Davies, A. W. & Moore, T. (1948). *Biochem. J.* **42**, lxiii.
Deshmukh, D. S., Malathi, P. & Ganguly, J. (1964). *Biochem. J.* **90**, 98.
Dowling, J. E. & Wald, G. (1958). *Proc. nat. Acad. Sci., Wash.,* **44**, 648.
Drummond, J. C., Bell, M. E. & Palmer, E. T. (1935). *Brit. med. J.* i, 1208.
Eden, E. & Sellers, K. C. (1950). *Biochem. J.* **46**, 261.
Fisher, L. R. & Kon, S. K. (1959). *Biol. Rev.* **34**, 1.
Fisher, L. R., Kon, S. K. & Plack, P. A. (1957). *Proc. roy. Soc.* B, **147**, 200.
Friend, C. J., Heard, C. R. C., Platt, B. S., Stewart, R. J. C. & Turner, M. R. (1961). *Brit. J. Nutr.* **15**, 231.
Futterman, S. & Andrews, J. S. (1964). *J. biol. Chem.* **239**, 4077.
Glover, J. & Morton, R. A. (1948). *Biochem. J.* **42**, lxiii.
Gray, E. L., Morgareidge, K. & Cawley, J. D. (1940). *J. Nutr.* **20**, 67.
Kramer, B., Sobel, A. E. & Gottfried, S. P. (1947). *Amer. J. Dis. Child.* **73**, 543.
Krinsky, N. I. & Ganguly, J. (1953). *J. biol. Chem.* **202**, 227.
Lewis, J. M., Bodansky, O., Birmingham, J. & Cohlan, S. Q. (1947). *J. Pediat.* **31**, 496.
McCance, R. A. & Widdowson, E. M. (1960). *Spec. Rep. Ser. med. Res. Coun., Lond.,* no. 297.

Mahadevan, S. & Ganguly, J. (1961). *Biochem. J.* **81**, 53.
Mahadevan, S., Seshadri Sastry, P. & Ganguly, J. (1963a). *Biochem. J.* **88**, 531.
Mahadevan, S., Seshadri Sastry, P. & Ganguly, J. (1963b). *Biochem. J.* **88**, 534.
Ministry of Agriculture, Fisheries and Food: National Food Survey Committee (1964). *Domestic Food Consumption and Expenditure :* 1962. London: H.M. Stationery Office.
Murray, T. K., Stainer, D. W. & Campbell, J. A. (1959). *Canad. J. Biochem. Physiol.* **37**, 1469.
National Research Council (1964). *Publ. nat. Res. Coun., Wash.,* no. 1146.
Plack, P. A. (1959). *Brit. J. Nutr.* **13**, 111.
Plack, P. A. (1963). *Brit. J. Nutr.* **17**, 235.
Plack, P. A. & Kon, S. K. (1961). *Biochem. J.* **81**, 561.
Popper, H. & Greenberg, P. (1941). *Arch. Path. (Lab. Med.),* **32**, 11.
Popper, H. & Volk, B. W. (1944). *Arch. Path. (Lab. Med.),* **38**, 71.
Rosenheim, O. & Webster, T. A. (1927). *Biochem. J.* **21**, 111.
Shantz, E. M. & Brinkman, J. H. (1950). *J. biol. Chem.* **183**, 467.
Thompson, S. Y., Braude, R., Coates, M. E., Cowie, A. T., Ganguly, J. & Kon, S. K. (1950). *Brit. J. Nutr.* **4**, 398.
Thompson, S. Y., Ganguly, J. & Kon, S. K. (1949). *Brit. J. Nutr.* **3**, 50.
Winterstein, A. & Hegedüs, B. (1960). *Hoppe-Seyl. Z.* **321**, 97.

Chemical structure and vitamin A activity

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Investigations on the relationship between chemical structure and vitamin A activity have a long, and at times somewhat desultory, history. The traditional approach has been to take experimental animals on a vitamin A-deficient diet and give them the test substance to see if it supports growth. Some of the compounds thus found to be active are substances containing the retinol (vitamin A_1 alcohol) structure (Fig. 1) within their own much larger molecule, e.g. carotenoids; these provitamins have been dealt with by S. Y. Thompson (1965). This paper is concerned with substances having approximately the same chain length as that of retinol.
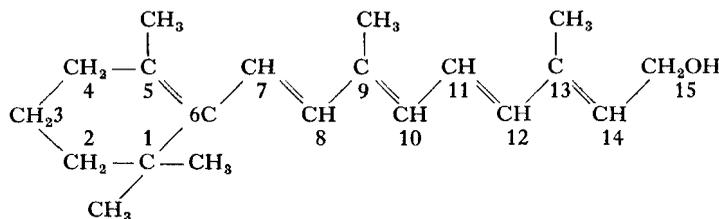

Fig. 1.   Structural formula of retinol (vitamin A_1 alcohol).

The results of work on many such substances have been reviewed by Isler (1950), Baxter (1952, 1963), Milas (1954), Moore (1957) and Heilbron & Weedon (1958). They can be summarized in the generalization that any change in the retinol molecule, except on the terminal (15) carbon atom, considerably reduces growth-promoting power. For example, biological activity is completely eliminated by modifications such as the shift of the ring double bond to the 4-position, out of conjugation with the side-chain, giving α-retinol (α-vitamin A) (Ames, Swanson & Harris, 1955), the saturation of a side-chain double bond (Gould, 1936), alterations in the methyl groups (Cheeseman, Heilbron, Jones, Sondheimer & Weedon, 1949) or shortening of the side-chain (Dowling, 1961; Baxter, 1963).