**Relation between the Quality of Canned Fish and
Its Content of ATP-breakdown—III.
ATP-breakdowns in Canned Albacore and Skipjack in
Relation to the Organoleptic Inspection***

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The ratios of Hx and IMP content to total Hx, HxR and IMP content in canned albacore and skipjack meat were measured and correlated with organoleptic judgments by Japanese and American inspectors in order to establish a useful chemical index of meat "decomposition". A linear relation expressed by a simple equation and a high correlation coefficient (over 0.9) between the Hx or IMP ratios in raw and canned meat was obtained. As the inspection scores (odour judgments by Japanese inspectors) pass from high rating a' through b, b' down to low ratings C and D, there is a definite increase in the Hx ratios of the respectively rated canned meats. From this, it was inferred that the Japanese inspection standard of odour is closely related to the freshness of fish material. The Japanese inspection scores based on odour and the American inspection judgments of "decomposition" of canned albacore and skipjack prepared from frozen fish agreed fairly well with each other. There was, however, a pronounced disagreement in inspection judgements of canned "winter albacore" and canned skipjack prepared from iced fish.

The quality evaluation of the canned foods is at present mainly based on the results of organoleptic inspection, but if the standard of organoleptic inspection can be supported by objective values, for example, the values of chemical analysis, the practice of the inspection will be considerably facilitated. It is already reported by many researchers¹⁻⁹⁾ that the amounts of hypoxanthine (Hx), inosine (HxR), and inosinic acid (IMP), which are the main products of ATP-breakdowns in the fish meat, are reliable indicators of the freshness of raw fish. It has been also well known that the quality of canned fish is greatly influenced by the freshness of raw fish. HUGES¹⁰⁾ and CRAWFORD¹¹⁾ have reported with herring and tunas respectively the existence of a linear correlation between the amounts of Hx in raw meat and canned meat. They also recognized that with the increase of Hx in the canned meat, the scores of their organoleptic judgement declined. In the previous papers,¹²⁻¹⁴⁾ the authors have reported already that the ratio of Hx or IMP amount to the total amount of Hx, HxR, and IMP in canned meat is an useful chemical index for evaluation of the quality of canned salmon, trout, and mackerel.

In recent times a considerable amount of Japanese canned albacore and skipjack

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exported to the United States was rejected of entry in the market there on the ground of the "decomposition" of meat, causing a serious damage to the parties concerned. This has made us suspect if there be wide discrepancy in the inspection standard between Japan and America. To straighten out this problem, a team of four FAD inspection experts came over to Japan in January 1972 for joint conference with the inspectors of the Japan Canned Food Inspection Association. During this conference, over 60 canned albacore and skipjack were organoleptically inspected jointly by the inspectors of both countries. To solve the problem of the organoleptic judgement, which is likely to be affected by subjective factors, it is necessary to employ more objective values such as the value of chemical analysis as index and to bring the organoleptic judgement of both countries to a common standard.

Incidentally different standards are used for the quality control of canned fish between Japan and America. The American standard puts more weight on the freshness of the raw fish, while the Japanese on the odour of canned fish. Therefore, if the relation between the judgement of the products by the odour and those by the freshness of the raw fish can be clearly defined, this will make a good step forward to the mutual understanding of the inspectors of the two countries. In the present paper, the results of the ATP-breakdowns in canned albacore and skipjack measured in relation to the organoleptic judgements by the Japanese and American inspectors to establish feasible chemical index of the "decomposition" of meat are discussed.

Materials and Methods

Material fish: Were used as material fish frozen and iced albacore and iced skipjack. The frozen albacore was caught in April 1971 in the neighboring seas of the Galapagos, frozen aboard at -45°C , and stored at -35 – -45°C for about 7 months. The iced albacore was caught in the western seas of the Northern Pacific in December 1971, hence of the so-called "winter albacore", and iced aboard for about 2 weeks. The iced skipjack was caught in the seas situated 137–142 E., 2–5 N. and iced aboard for about 2 weeks. The frozen albacore weighed about 14–21 kg, the iced one about 12–13 kg, and the skipjack about 3–5 kg. The material fish were all placed in tanks with running water at 16.5°C , and taken out of the tank at intervals of about 24 hr. They were then put to the usual canned manufacturing process.

Samples of canned fish: Material fish were cooked at 102°C for 220 min after the usual process, and sterilized at 113°C for 90 min after filling in cans. For comparison were taken 7 cans each from classes 1, 2, and 3 of the American canned albacore and skipjack prepared from frozen fish, which were manufactured in Terminal Island, Los Angeles, California in December 1971. The frozen fish were classified organoleptically

into classes 1, 2, and 3 by the American inspector, according to the degree of freshness, which correspond to the expressions "fishy", "stale", and "taint".

Analysis of Hx, HxR, and IMP: For the analysis of Hx, HxR, and IMP, 2 g each of the ordinary meat were taken from 4 different parts of the material fish, that is from the central part of the back and belly side, and the fore and rear parts on the lateral line. The meat and juice of each sample of canned fish were thoroughly mixed after the organoleptic inspection, and 2 g each were taken and analyzed. Furthermore, about 10 g each of the ordinary meat were taken from the central part of the back side of 9–10 frozen albacore of classes 1, 2, and 3 mentioned above in America, and the extract of perchloric acid was obtained as follows.

To the sample meat is added 25 ml of 10% cold perchloric acid solution. The mixture is stirred, left to stand for about 30 min for extraction, and then centrifuged ($2,800 \times g$ for 20 min). The residue is extracted again by the same process. The extract is neutralized to pH 7.6, and the supernatant after centrifugation ($1,700 \times g$ for 10 min) is made to 100 ml with distilled water and submitted to the analysis of Hx, HxR, and IMP. In case of canned fish in oil, instead of mixed meat, 2 ml of the juice from which the floating oil was previously removed is used for the analysis.

As shown in Table 1, 4 test tubes containing 1 ml of extract and 2 ml of buffer solution are prepared, and 1.5 or 2 ml of distilled water and 0.5 ml each of enzyme solutions

Table 1. Compositions of reaction systems.
Tubes for the measurement of Hx, HxR, and IMP

Tube No.	Extract of meat	Buffer solution		Distilled water	Enzyme solution
1	1 ml	Phosphate buffer	2 ml	2 ml	—
2	1 "	" "	2 "	1.5 "	A. 0.5 ml
3	1 "	" "	2 "	1.5 "	B. 0.5 "
4	1 "	NH ₄ Cl-NH ₄ OH buffer	2 "	1.5 "	C. 0.5 "

Tubes for the measurement of enzyme solution blank

Tube No.	Extract of meat	Buffer solution		Distilled water	Enzyme solution
1'	—	Phosphate buffer	2 ml	3 ml	—
2'	—	" "	2 "	2.5 "	A. 0.5 ml
3'	—	" "	2 "	2.5 "	B. 0.5 "
4'	—	NH ₄ Cl-NH ₄ OH buffer	2 "	2.5 "	C. 0.5 "

Note: After enzymatic reaction, the absorbances at $290 \text{ m}\mu$ at $290 \text{ m}\mu$ of the tubes from 2 to 4 were measured using the tube 1 as reference, and likewise those of the tubes from 2' to 4' were measured using the tube 1' as reference. The phosphate buffer was a mixture of M/15 sodium phosphate dibasic solution and M/15 potassium phosphate monobasic solution and pH is 7.6. The ammonium chloride-ammonia buffer was a mixture of M/10 ammonium chloride solution and M/10 ammonia water with pH 9.5, to which was added 0.2% sodium phosphate dibasic 12 hydrate.

A, B, and C is added. The contents of each tube are thoroughly stirred and placed for 40 min at 37°C to allow enzymatic reaction to proceed. After the reaction, their absorbances at 290 m μ are measured. From the values thus measured are subtracted the values of the enzyme blank respectively, and the amounts of Hx, HxR, and IMP are calculated based on the equation derived from the calibration curve of the standard reagents. If the absorbances at 290 ml of the tubes 2 to 4 shown in Table 1 are expressed as E290 A, E290 B, and E290 C, and those of the enzyme blank tubes as E290 a, E290 b, and E290 c, the amounts (μ mole) of Hx, HxR, and IMP in 1 g of sample meat can be calculated by the following equations:

$$\text{Hx} = 0.401 \times (\text{E290A} - \text{E290a}) \times 100/\text{g of sample}$$

$$\text{HxR} = 0.401 \times \{\text{E290B} - \text{E290A} - (\text{E290b} - \text{E290a})\} \times 100/\text{g of sample}$$

$$\text{IMP} = 0.401 \times \{\text{E290C} - \text{E290B} - (\text{E290c} - \text{E290b})\} \times 100/\text{g of sample}$$

In the present paper, the ratios of the amounts of Hx and IMP to the total amount of Hx, HxR, and IMP were used as the indices of the freshness of material fish or the quality criteria for canned fish.

With the enzyme solutions A, B, and C, 0.03 unit of xanthine oxidase, 0.03 unit of xanthine oxidase and 0.5 μ g of nucleoside phosphorylase, and 0.03 unit of xanthine oxidase, 2.5 μ g of nucleoside phosphorylase, and 100 μ g of alkaline phosphatase (type II) exist respectively. From this it follows that Hx in the tube 2, Hx and HxR in the tube 3, and Hx, HxR, and IMP in the tube 4 are all converted into uric acid, and corresponding to the amount of uric acid formed, their absorbances at 290 m μ are increased.

In the experiments were used xanthine oxidase of Worthington Biochemical Co., and nucleoside phosphorylase and alkaline phosphatase (type II) of C.F. Beohringer Co.

Organoleptic judgement of the canned fish: Among the many check-points of inspection applied to the canned albacore and skipjack exported from Japan was chosen only the odour, which directly concerned the judgement of the "decomposition" of meat, by the inspectors of the Japan Canned Foods Inspection Association for the organoleptic inspection. The scoring was classified into a, a', b, b', C and D—6 classes in all. Only Those products are exported, which belong to classes a and a' (inclusive of those which fall within the tolerable limits of b or b') are exported to the United States. These export canned fish are called "Fancy A".

The American inspector, together with the Japanese inspectors, made a joint inspection on a part of the samples of canned fish, and evaluated the odour of the meat. American inspector's decisions of the organoleptic judgement were expressed as "good" (not "decomposition") and "bad" ("decomposition").

Results and Discussions

Changes of the ratios of Hx and IMP in raw meat: The IMP is accumulated considerably in the meat after the death of the fish as a result of rapid decomposition of ATP and ADP, and gradually decreases with the decomposition into HxR by phosphatase in the meat. Hx is hardly detectable in the meat right after the death, but it is produced from HxR by ribosidase or nucleosidase in the meat, and increases with the standing time of raw fish, accompanying the simultaneous decrease of IMP.

Changes of the ratios of Hx and IMP in the meat of frozen albacore held in tank with running water 16.5°C are shown in Fig. 1. The ratio of IMP in the meat rapidly decreases with elapsed holding time in the tank, while the ratio of Hx increases corresponding with the decrease of IMP ratio. In comparison with those two ratios in a definite standing time, the ratio of IMP changes more swiftly than the Hx ratio in the early stage and in the later stage the ratio of Hx more rapidly than the IMP ratio. From this

Fig. 1. Ratios of Hx and IMP to the total ATP-breakdowns, measured on the meats of frozen albacore held in tank with running water at 16.5°C for varying periods.

Note: The vertical bar on each average value shows the fluctuation of values.

×---× Hx   ●---● IMP

result the ratio of IMP in the early stage of the storage and the ratio of Hx in the later stage are found to be good indices by which the freshness of raw fish is determined. But considering from the result of the preliminary examination that the Hx ratios of the meats in the "decomposition" cans were all over 20% and the freshness of raw fish many have been in the later stage, we have taken the Hx ratio as more reliable index for the "decomposition", and used mainly it as a yardstick in the discussions of all subsequent experiments.

Relation between the ratios of Hx and IMP in the raw meat and canned meat: The correlation of the ratios of Hx and IMP between the raw meat and canned meat of frozen albacore are shown in Figs. 2 and 3. The frozen albacore were left for 116 hr in a tank

Fig. 2. Relation between the ratios of Hx in the raw meat and canned meat from frozen albacore held in tank with running water at 16.5°C for varying periods.

Note: The vertical and horizontal bars on each point show the fluctuation of values measured on the raw meat and canned meat.

Fig. 3. Relation between the ratios of IMP in the raw meat and canned meat from frozen albacore held in tank with running water at 16.5°C for varying periods.

Note: The vertical and horizontal bars on each point show the fluctuation of values measured on the raw meat and canned meat.

with running water at 16.5°C. As the Hx and IMP are very heat-stable¹⁶⁻¹⁷⁾, a substantial part (approximately over 90%) of the Hx and IMP in raw meat will be retained in the canned meat. Therefore, as shown in Figs. 2 and 3, between the ratios of Hx and IMP in the raw meat (Y) and canned meat (X), there exist a linear relation and a high correlation coefficient (r_{XY}) of 0.955, 0.915 which are expressed by the regression equations $Y=0.885X+2.00$, $Y=0.800X+7.15$, calculated from the mean value of each group. From this result it follows that if the ratios of Hx and IMP are known either in

the raw meat or canned meat, the ratios in the other can be inferred with high accuracy. For canned tunas, CRAWFORD¹¹⁾ reported also the existence of linear relations between Hx amounts in the raw meat (Y) and canned meat (X) in albacore, bluefin, and yellowfin, which are respectively $Y=0.737X+2.43$, $Y=1.012X+1.44$, and $Y=0.813X+2.03$.

Fig. 4. Relation between the ratios of Hx in the raw meat and canned meat from frozen albacore classified into classes 1, 2, and 3 by the degree of freshness in America.

● in brine ○ in oil

The relation between Hx ratios in the raw meat and canned meat of albacore prepared in the United States is shown in Fig. 4. The data on the left of the figure represent the analytical results of the raw meat and those on the right those of the canned meat. Unfortunately as it was unascertainable whether each sample can was prepared from the same raw fish that was analyzed, it was impossible to construct a correlation graph as shown in Fig. 2. But as the Hx ratios in the raw meat and canned meat of each class

present very similar values, it is quite conceivable that there may exist evidently a high correlation between them analogous to those in Fig. 2. The similar high correlation as in the case of Hx ratios is also observed between the IMP ratios in the raw meat and canned meat.

Relation between the Hx ratio in canned albacore meat and the organoleptic judgement: The relations between the Hx ratios in canned meat prepared from frozen albacore in Japan and America and their organoleptic judgements by the odour are shown in Figs. 5 and 6, and also the relation between them on the sample cans prepared from

Fig. 5. Relation between the Hx ratios and the organoleptic judgements by the odour in the canned meats prepared from frozen albacore in Japan.

Good: not "decomposition"

Bad: "decomposition"

the "winter albacore" is shown in Fig. 7. For convenience of comparison, the inspection scores judged by the Japanese inspectors and the judgement on the "decomposition" by the American inspector are shown on the same figures, the former on the left and the latter on the right.

As the inspection scores judged by the odour made by the Japanese inspectors pass from high rating a' through b, b' down to C and D, the ratios of Hx in the canned meat

Fig. 6. Relation between the Hx ratios and the organoleptic judgements by the odour in the canned meats prepared from frozen albacore in America.

Good: not "decomposition" ● in brine
Bad: "decomposition" ○ in oil

of each rating clearly indicate a definite increase. As seen in Fig. 5 were the largest number of cans were inspected, there are significant variance with $p=0.01$ and 0.05% between the Hx ratios of a' and b , and b and b' . These findings suggest the existence of an extremely high correlation between the inspection scores judged by the odour and the Hx ratio in the canned meat, in other words, the degree of freshness of the raw fish, and lead to the well-founded inference that the standard of judgement by the odour made by the Japanese inspectors is closely related to the freshness of raw fish. With the canned "winter albacore" presented in Fig. 7, there is only a slight increase in the average of the Hx ratio of each rating, as the Japanese inspection scores judged by the odour shift from high rating a' through b down b' . From this it is guessed that the standard of the Japanese inspection scores of the canned "winter albacore" is more stringent than that of the canned frozen albacore.

Furthermore, with the canned albacore prepared from the frozen fish shown in Figs.

Fig. 7. Relation between the Hx ratios and the organoleptic judgements by the odour in the canned meats prepared from iced "winter albacore" in Japan.
 Good: not "decomposition"
 Bad: "decomposition"

5 and 6, the judgement on the "decomposition" by the American inspector and the Japanese inspection scores judged by the odour agree fairly well with each other. The American rating "good" corresponds to the Japanese rating a' and b, and "bad" to C and D. Moreover, though it is not indicated on the figures, what was judged as "passable" by the American inspector was found to correspond to b' rating. In Fig. 7, however, though the rating "good" of the canned "winter albacore" approximately falls in with the ratings a' and b, the rating "bad" dispersed over a wide range from a' to C. In this connection the authors would point out that it is known from old in this country that the "winter albacore" has a specific odour popularly called the "winter albacore odour". It is very likely that the American inspector was strongly swayed by this specific odour in his organoleptic judgement on the canned "winter albacore".

Relation between the Hx ratio in canned skipjack meat and the organoleptic judgement: The relations between the Hx ratios in the canned skipjack meats prepared in Japan and America and their organoleptic judgements by the odour are shown in Figs. 8 and 9. As in the case of canned albacore, the Hx ratio in the canned skipjack meat constantly increases as the Japanese inspection scores judged by the odour shift from the high to the lower ratings. This indicates that the freshness of raw fish is reflected

Fig. 8. Relation between the Hx ratios and the organoleptic judgements by the odour in the canned meats prepared from iced skipjack in Japan.

Good: "not decomposition"

Bad: "decomposition"

on the standard of the Japanese inspection scores of the canned skipjack. The American judgement on the "decomposition" of canned skipjack prepared from frozen fish, plotted in Fig. 9, agrees fairly well with the Japanese inspection scores judged by the odour, presenting a similar pattern as the results of canned albacore shown in Figs. 5 and 6, namely "good" corresponding to a', passable to b, b' and "bad" to C and D, respectively. With the canned skipjack prepared from iced fish shown in Fig. 8, however, the situation is a

Fig. 9. Relation between the Hx ratios and the organoleptic judgements by the odour in the canned meats prepared from frozen skipjack in America.

Good: not "decomposition" ● in brine

Bad: "decomposition" ○ in oil

little different. While the rating "good" approximately agrees with a' and b as shown in Fig. 9, the rating "bad" does not quite agree with the Japanese rating, as the Hx ratio disperses over a wide range from a' to D, though the number of sample cans tested was only three and too few to attain any decisive conclusion.

With the canned skipjack, the Japanese and American inspections do not seem to come up to a complete agreement on the standard of judgement on rancidity and similar odour. But, at the recent joint inspection conference, the number of canned skipjack, suspected of having rancidity and similar odour, was too small to give any conclusive data.

Comparison of the organoleptic judgements between Japan and America: From the results of the canned albacore prepared from frozen fish shown in Figs. 5 and 6, it can be seen that what was judged as "bad" by the American inspector would have a Hx

ratio of about 38% at the lower limit, and high enough to be rated C and D by the Japanese inspectors. In other words, the canned frozen albacore accepted by the Japanese inspection could not have been judged by the American inspection as "bad". But the situation would be different with the canned "winter albacore" presented in Fig. 7. As the lower limit of "bad" in the American judgement is a Hx ratio of about 11%, evidently there is a possibility that a large quantity of the canned "winter albacore" accepted by the Japanese inspection standard which is stricter than the standards shown in Figs. 5 and 6 may be rejected of entry in America as "bad".

To straighten out this large discrepancy in the organoleptic judgement on canned "winter albacore" between Japanese and American inspectors, it is highly recommendable that the United States authorities should reconsider the present standard of judgement on the "decomposition" practised by the American inspector and base it on the freshness of the raw fish as proposed by America herself, so that the standard would be mutually acceptable to both countries.

Therefore, judging from the scores of canned skipjack prepared from frozen fish given in Fig. 9, if the American rating "bad" is expressed by the Hx ratio, it is 21% at the lower limit, and will correspond to the Japanese ratings C and D, which lead us to infer that the canned skipjack passed as "acceptable" by the Japanese inspectors will not be rejected as "bad" by the American inspector. With regard to the canned skipjack prepared from iced fish, however, as the lower limit of "bad" in the American judgement is a Hx ratio of about 9%, there is a danger of the canned skipjack accepted by the Japanese inspectors being rejected by the American inspector as "bad". In this connection also, it is highly desirable that the standards of judgement of both Japan and America should be adjusted by basing it on the freshness of raw fish as in the case of the canned "winter albacore".

The relation between the Japanese inspection scores and the American judgement on the canned albacore and skipjack is presented in Figs. 10 and 11. On the differences between the Japanese and American inspection standards, already discussed in the foregoing sections, those of the canned "winter albacore" are shown in Fig. 10, and those of the canned skipjack prepared from iced fish in Fig. 11. Apart from these differences, the organoleptic judgements by the Japanese and American inspectors show very good agreement among them, the rating "good" corresponding to a' or b, "passable" to b' and "bad" to C in the canned albacore, while similarly in the canned skipjack "good" to a', "passable" to b or b' and "bad" to C and D, and there is practically no exception to it.

In Table 2 are summarized the inspection scores judged by the odour made by the Japanese inspectors and the judgement on the "decomposition" by the American inspector in comparison with the mean values of Hx and IMP ratios in the canned meat of each rating. In the canned albacore, the rating b' and "bad" of the canned "winter albacore"

Fig. 10. Relation between the Japanese inspection scores judged by the odour and the American judgements on the "decomposition" of canned albacore.

Good: not "decomposition"

Passable: not "decomposition" but low quality

Bad: "decomposition"

● Sample cans prepared from frozen albacore in Japan

× Sample cans prepared from frozen albacore in America

○ Sample cans prepared from iced "winter albacore" in Japan.

Fig. 11. Relation between the Japanese inspection scores judged by the odour and the American judgements on the "decomposition" of canned skipjack.

Good: "not decomposition"

Passable: not "decomposition" but low quality

Bad: "decomposition"

● Sample cans prepared from iced skipjack in Japan

× Sample cans prepared from frozen skipjack in America

show 7–11% higher IMP ratio and 17–22% lower Hx ratio than the canned frozen albacore. From the results a fact is suggested that the specific odour of the "winter albacore" strongly affects the organoleptic judgement. Furthermore, in the rating "bad" of canned frozen albacore, the IMP ratio is 2–4% lower and Hx ratio 1–4% higher than the rating C, while the rating "bad" of the canned "winter albacore" shows about 2% higher IMP ratio and about 4% lower Hx ratio than the rating C. Such being the analytical findings, it is very possible that the American standard of judgement on the "decomposition" of the canned "winter albacore" was evidently more stringent than that of the Japanese inspection scores judged by the odour.

With regards to the canned skipjack, though the rating "bad" of the canned skipjack prepared from frozen fish in America shows 1% lower IMP ratio and 5% higher Hx ratio than the rating C, the "bad" rating of the canned skipjack prepared from iced fish shows 2% higher IMP ratio and 5% lower Hx ratio than C rating, evidencing a considerable discrepancy between Japan and America in their standard of organoleptic judgement.

Table 2. Relation between the ratios of Hx and IMP in canned meat and the organoleptic judgement on three kinds of canned albacore and two kinds of canned skipjack prepared for the present experiment.

Kind of canned fish	Japanese inspection scores judged by the odour	American judgements on the "decomposition"	Number of sample cans	Hx ratio*	IMP ratio*
canned	a'		51	17.5	38.4
albacore	b		13	28.0	23.6
prepared	b'		9	36.1	16.6
from frozen	C		7	39.2	15.3
fish in Japan	D		5	41.4	11.9
		"Good"	14	20.3	32.0
		"Bad"	4	40.6	12.9
Canned	a'		8	15.0	44.7
albacore	b		4	26.4	26.9
prepared	b'		5	40.3	14.5
from frozen	C		4	44.9	14.6
fish in America		"Good"	6	22.2	35.1
		"Bad"	3	44.6	10.7
Canned	a'		23	14.8	34.8
albacore	b		7	18.2	27.3
prepared	b'		7	19.0	24.4
from iced	C		8	36.2	13.6
"winter alba- core" in Japan		"Good"	7	14.3	31.6
		"Bad"	8	23.0	22.3
Canned	a'		10	14.1	23.0
skipjack	b		8	19.7	21.4
prepared	b'		7	26.0	16.3
from iced	C		8	32.3	15.3
skipjack	D		4	37.0	11.4
in Japan		"Good"	9	19.9	20.8
		"Bad"	3	27.4	16.9
Canned	a'		7	12.7	20.6
skipjack	b		2	18.1	20.8
prepared	b'		2	25.2	17.5
from frozen	C		3	24.5	12.7
fish in America	D		7	35.9	13.3
		"Good"	4	14.8	21.7
		"Bad"	5	29.9	11.7

* Average values.

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